



## The allopatric phase of speciation: the sharp-beaked ground finch (*Geospiza difficilis*) on the Galápagos islands

PETER R. GRANT\*, B. ROSEMARY GRANT AND KENNETH PETREN

*Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544-1003, U.S.A.*

*Received 13 January 1999; accepted for publication 6 July 1999*

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Using microsatellite DNA variation, morphological measurements and sonagrams made from tape-recordings in the field, we examine the allopatric differentiation of six populations of the sharp-beaked ground finch, *Geospiza difficilis*, in the Galápagos archipelago. We ask how and why the populations became differentiated, and consider what the differences imply about speciation. An important factor is time; *G. difficilis* is one of the phylogenetically oldest species. Populations became differentiated by adapting in beak morphology to different food supplies. Since beak size and shape also function in conspecific mate recognition and choice, the potential for reproductive isolation from sister and parental taxa arose as a correlated effect of natural selection on resource-exploiting traits. This conforms to a standard explanation for the origin of pre-mating isolation as a byproduct of adaptive changes in allopatry without being adaptive itself. However, this explanation is incomplete. Vocal characteristics also differentiated, partly as a result of natural and sexual selection independent of beak size change in environments with different sound transmitting properties. An additional element of chance is indicated by a comparison of closely-related populations on two islands, Wolf and Darwin, that are geographically close, and topographically and floristically similar. The populations have markedly different vocalizations. Morphological characters, vocalizations and genetic traits do not vary concordantly among the six populations. One population (Genovesa) is genetically more similar to other congeneric species. Phenotypic similarities with *G. difficilis* are the result of a uniquely long retention of shared ancestral traits. Arguments under the phylogenetic species concept justify recognizing this population as a separate species, *Geospiza acutirostris*. Under the biological species concept it should remain as currently classified, *G. difficilis*.

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ADDITIONAL KEY WORDS:—microsatellite DNA – phylogeny – beak shape – vocalizations – hybridization – cultural evolution – pre-mating isolation – adaptation – chance.

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\* Corresponding author. E-mail: [prgrant@princeton.edu](mailto:prgrant@princeton.edu)

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## INTRODUCTION

Speciation is usually a protracted process of differentiation that eventually leads to the complete cessation of genetic exchange between two populations derived from a common ancestor (Dobzhansky, 1937, 1940; Mayr, 1940, 1942). Intermediate stages include formation of two allopatric or parapatric populations from one, differentiation through adaptive and/or nonadaptive processes, and a sympatric phase with or without interbreeding. Recent research has largely concentrated on the sympatric phase to document the genetical, ecological and behavioral differences between the incipient species (Hollocher, 1998; Losos, 1998; Schluter, 1998), and to understand how the differences are possibly enhanced or reinforced by selection (Liou & Price, 1994; Noor, 1997; Sætre *et al.*, 1997; Coyne & Orr, 1998), and affected by hybridization (Arnold, 1992; Grant & Grant, 1992).

Leaving aside the fact that differences between incipient species may arise entirely in sympatry (Bush, 1994; Guldmond & Dixon, 1994; Schliewen *et al.*, 1994; Asquith, 1996; Feder, 1998), our concern here is with the allopatric phase in the speciation process, i.e. those situations where a species is polytypic, with allopatric populations differentiated in morphology. The populations may forever stay separated in space and weakly differentiated, or they might be in the process of diverging to the point of being incapable of exchanging genes at a later time of establishing parapatry or sympatry. It has not been possible to formulate any general principles that would allow prediction of these alternative fates (Foster *et al.*, 1998). Mayr (1969) posed the problem by stating that “In the same period during which one isolate may have hardly subspeciated, the isolate of another species in the same refuge may have reached full species level” (p. 16), and concluded “Our ignorance is still complete when it comes . . . to an answer for the question why certain populations in an isolate participate in a speciation while others do not.” (p. 17).

Factors which promote allopatric differentiation include the degree of ecological difference and hence selection regimes, population size and hence the potential role of drift, and time. They have rarely been measured or estimated. They are likely to vary in relative importance among organisms and environments, which is why no stronger statement of generality can be made. Perhaps the least understood is how and why pre-mating isolation evolves, and the role of sexual selection (Carson, 1997; Price, 1998).

A favourable situation for investigating all of these factors is an adaptive radiation of organisms that show various stages in the speciation process (e.g. see Wagner &

Funk, 1996; Givnish & Sytsma, 1997; Foster *et al.*, 1998; Grant, 1998). Darwin's finches are one of the most suitable because they have been relatively unaffected by human-caused extinctions. Fifteen species, represented by a number of populations varying from one to more than a dozen, occur on the Galápagos islands and Cocos Island (Grant, 1986; Petren *et al.*, 1999a). Ecological differentiation has been well investigated at species and population level. At both levels variation in diets is adaptively related to variation in beak morphology (Schluter & Grant, 1984a,b; Grant, 1986; Grant & Grant, 1989). Individuals of sympatric species make mating discriminations on the basis of both song and morphological appearance when tested experimentally (Ratcliffe & Grant, 1983a, 1985), and mating patterns reflect the use of these two sets of cues (Grant & Grant, 1996a, 1997a,b). Behavioral repertoires used in courtship are remarkably similar among the species (Lack, 1945, 1947; Grant, 1986). These prior findings serve to focus attention on morphology and vocalizations, but especially the relatively unstudied vocalizations, as the main features of interest in the study of divergence of populations in allopatry.

We report differences in morphology and vocalizations among the most strongly differentiated group of conspecific populations; the sharp-beaked ground finch (*Geospiza difficilis*). Results are used to interpret the process of differentiation in allopatry as the first stage of speciation. To do this we need phylogenetic information to establish which species is related to which, and which gave rise to which. Until recently, comparative analyses of finch morphology and vocalizations had to be made in the context of a phylogeny based on allozymes (Yang & Patton, 1981; Polans, 1983). This phylogeny is poorly resolved however (Stern & Grant, 1996), and the level of resolution is species and not populations. A better supported phylogenetic reconstruction at species level, based on microsatellite allelic variation, is now available (Petren *et al.*, 1999a; see also Sato *et al.*, 1999, for a similar mtDNA phylogeny). Here we examine genetic relationships among *G. difficilis* populations and their closest relatives.

### *Geospiza difficilis*

The sharp-beaked ground finch (*G. difficilis*) was chosen as the focal species for two reasons. First, populations of *G. difficilis* represent different degrees of allopatric differentiation in the speciation process. They differ more in morphology than do those of any other species (Grant, 1986), and by so much that questions have been raised about their true affinities. Populations on the two northernmost islands of Wolf and Darwin (Fig. 1) are similar enough to *G. scandens* to have been considered members of this species when they were first discovered (Rothschild & Hartert, 1899). The population on Genovesa so closely resembles *G. fuliginosa* in body size (but not bill shape) that Vagvolgyi & Vagvolgyi (1989, 1991) have argued it should be considered a member of this species, whereas Lack (1945, 1947) concluded it could not be distinguished even at subspecific level from the population on Pinta (see also Schluter *et al.*, 1991). Difficulties of species determination are to be expected when speciation involves morphological divergence of allopatric relatives. Populations like these are often referred to as a superspecies (Mayr, 1942; Lack, 1945).

Second, populations of this species differ ecologically to an extent unrivalled among the finches (Schluter & Grant, 1984a; Grant, 1986). Three occur on small, low, arid, islands and three occur at high elevation in mesic habitat on high and

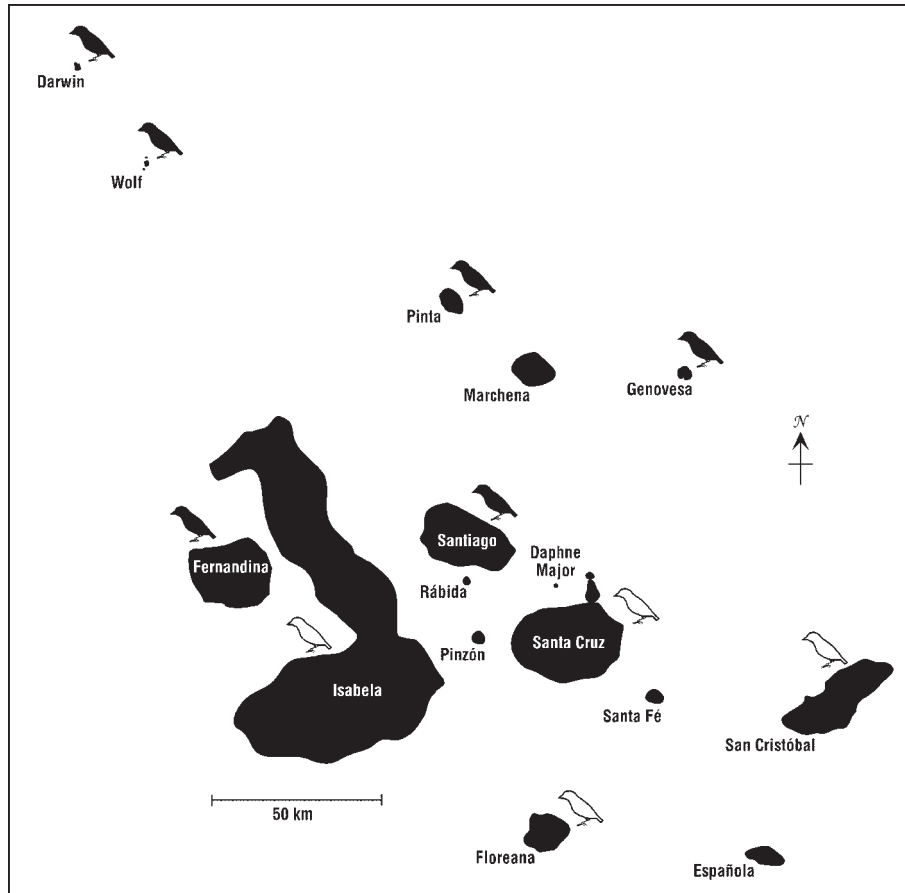


Figure 1. Map of the Galápagos, showing extant (solid symbols) and extinct (open symbols) populations of *Geospiza difficilis*. It is possible that one or more populations still exist on the volcanoes of Isabela (see also Table 1).

large islands (Fig. 2, Table 1). The low-lying islands of Wolf and Darwin are covered by *Croton scouleri* (a shrub), *Opuntia* cactus, grasses and a few species of low herbs. The low-lying island of Genovesa has a similar but richer plant community, and is covered by a forest of drought-deciduous trees, mainly *Bursera graveolens*, *Cordia lutea* and *Croton scouleri*. Populations of *G. difficilis* on the other three islands, Fernandina, Santiago and Pinta, live at high altitude in *Zanthoxylum fagara* forest. Forest cover is continuous over large areas of all islands except Wolf and Darwin, yet there are open patches of vegetation on all of them too, occupied by different low-growing species on the different islands (Hamann, 1981; Schluter, 1982; Grant & Grant, 1989). Seeds are available on all islands at all times, but arthropods are more abundant year-round on the high islands than on the low islands.

#### Vocalizations

Adult Darwin's finches produce several types of vocalizations. These include structurally varied advertising songs, pure-tone whistles, wide-band frequency modulated buzzes, narrow-band buzzes, alarm notes or calls, and contact calls between

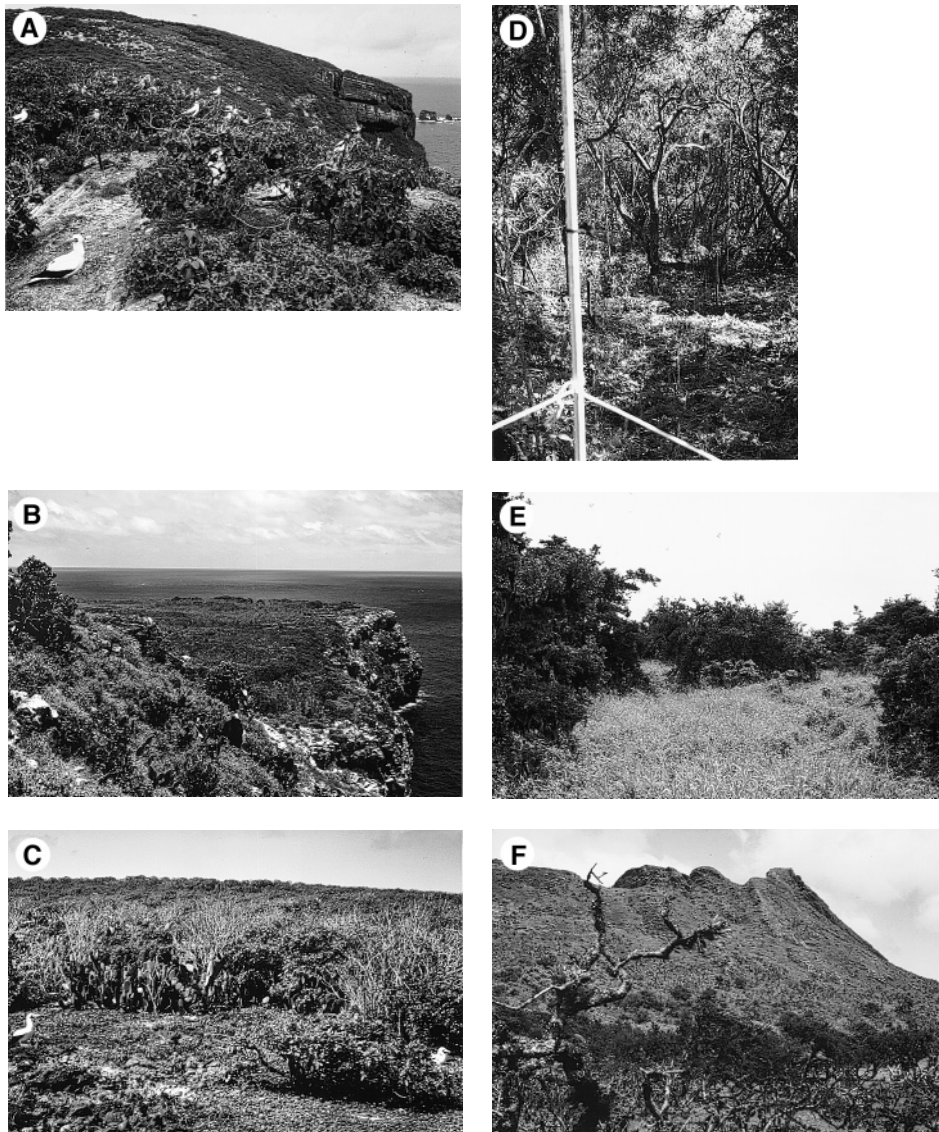


Figure 2. Contrasting habitats occupied by *Geospiza difficilis*. Low islands, left: (A) Darwin, (B) Wolf, (C) Genovesa. High islands, right: (D) Fernandina, (E) Pinta, (F) Santiago. The forest of *Zanthoxylum fagara* has been disturbed to some extent by introduced goats on Pinta, and widely destroyed by them on Santiago. Goats and other mammals (pigs, donkeys, etc.) have never been introduced to the other four islands. The Fernandina illustration shows a mist net station in mixed *Zanthoxylum-Scalesia* forest. Photographs by B.R. Grant (Fernandina, Pinta), P.R. Grant (Wolf, Genovesa) and M. Wikelski (Darwin, Santiago).

members of a pair (Bowman, 1979, 1983; Ratcliffe, 1981). We concentrate on songs, whistles and buzzes because these are all associated with courtship.

Songs of Darwin's finches are loud, relatively short (<2 s), and are sung by males,



TABLE 1. The distribution of *Geospiza difficilis* populations on Galápagos islands

Island	High or low	Habitat	Other finch species
Darwin	Low	<i>Croton–Opuntia</i>	4, 10
Wolf	Low	<i>Croton–Opuntia</i>	4, 10
Genovesa	Low	<i>Bursera–Cordia</i>	4, 5, 10
Fernandina	High	<i>Zanthoxylum</i>	1, 2, 3, 4, 6, 8, 10
Pinta	High	<i>Zanthoxylum</i>	1, 2, 3, 4, 6, 9, 10
Santiago	High	<i>Zanthoxylum</i>	1, 2, 3, 4, 6, 7, 8, 9, 10

Note. Other finch species: 1 *G. fuliginosa*; 2 *G. fortis*; 3 *G. scandens*; 4 *G. magnirostris*; 5 *G. conirostris*; 6 *Platyspiza crassirostris*; 7 *Cactospiza pallida*; 8 *Camarhynchus parvulus*; 9 *C. psittacula*; 10 *Certhidea olivacea* or *C. fusca*. Populations of *G. difficilis* formerly occurred in *Zanthoxylum* habitat at medium and high elevations on Santa Cruz, San Cristóbal, Floreana and Isabela (Grant, 1986), and have become extinct in the last 150 years owing to anthropogenic destruction of the habitat. It is not known if populations still exist on any of the volcanoes on Isabela.

unaltered, throughout life. Songs are used in long-distance communication; they attract mates as well as help to defend territories (and mates) against intruders. Song is a culturally transmitted trait. The repertoire is typically one song which is learned, usually from the father, during a period of parental dependence lasting for 30–40 days (Bowman, 1983; Grant & Grant, 1989, 1996a). Evidence from lab-reared birds suggests that daughters also learn their fathers' song (Bowman, 1979, 1983), and even though they do not sing they appear to base their choice of mate at least partly on song as revealed by mating patterns (Grant & Grant, 1996a, 1997a,b).

Whistles and buzzes are produced by males during bouts of singing advertising song, especially in the presence of females. Whistles are continuous or discontinuous (interrupted) pure tone sounds of up to three seconds or more duration (Bowman, 1983), produced at a narrow range of high frequencies or descending in frequency from about 16 kHz to 10 kHz or lower. Broad band buzzes are lower in amplitude and frequency. They have been labelled as special basic songs by Bowman (1979, 1983). Like whistles they are used to communicate information to potential and actual mates, as well as conspecific male intruders, over shorter distances and generally close to the nest (Bowman, 1983). Inheritance of whistle and buzz characteristics is unknown. A male *G. difficilis* from Wolf reared in captivity learned a whistle from a 'tutor' from Darwin, whereas a similarly reared male from Darwin did not learn the characteristic buzz vocalization from a Wolf tutor (Bowman, 1983).

The groundwork for the present study was laid by extremely comprehensive descriptive studies of vocalizations of Darwin's finches by Bowman (1979, 1983) and Ratcliffe (1981). We have extended these studies by recording vocalizations in a previously unstudied population of *G. difficilis* (Fernandina), and by recording vocalizations from many more males of the other five populations of this species than were available to them, as well as some populations of each of the remaining 14 species.

#### METHODS

Blood samples were obtained from all populations of *G. difficilis*, and several populations of all other species except for the rare mangrove finch, *Cactospiza heliobates*. Details of sampling, laboratory protocols for DNA extraction, genetic

screening for microsatellite variation, and statistical methods of analysis have been presented elsewhere (Petren, 1998; Petren *et al.*, 1999a,b). A species-level phylogeny was produced by combining microsatellite data at 16 loci (Petren *et al.*, 1999a,b). Nei's genetic distance measures were calculated, and species relationships were assessed by the UPGMA method. Here we use the same loci to examine relationships among individual populations. Sample sizes from two to six populations of each species are given in Petren *et al.* (1999a).

Morphological measurements were taken in an earlier study, reported and described by Schluter & Grant (1984a). Most were taken by D. Schluter. The full set of measurements are body mass (weight) in grams, wing length, tarsus length, and four bill measurements, all in mm; depth (height), width, total length and length to tip from a point along the commissure where beak depth is 4 mm. Univariate and multivariate analyses were performed on the measurements to characterize and compare the axes of morphological differentiation among six populations of *G. difficilis* and an additional two populations of *G. fuliginosa*. Principal components analysis was carried out on the correlation matrix, and Mantel tests were used to test for similarity of morphological and genetic matrices. Birds captured in mist nets were photographed.

Most of the vocalizations were recorded by us with Sony TC-D5 PRO II tape-recorders and Sennheiser directional microphones. In addition recordings were made on Santa Cruz and Wolf by David Day with a video Camcorder, on several Galápagos islands by Laurene Ratcliffe with a Uher Report-4100 recorder, and on Cocos Island by Tom Sherry with a Sony TCM 500. Sonagrams were made with a Kay Elemetrics Sonagraph. For most vocalizations the dynamic range was set at 25–35 decibels, but the low amplitude buzzes required a range of 45–60 dB. We have followed the terminology in Bowman (1983) except where indicated.

We define note as a continuous sound, appearing discretely on a sonagram. We use the term element to refer to different components of a note where the trace on the sonagram changes predictably without break in the continuity of the trace (sound). There is subjectivity in designating elements, so only one of us (PRG) classified them, and we have used them only to compare songs in broad categories. We consider a song to comprise one or more notes separated by a time interval shorter than a note length. Songs are considered to be sung in multiples if the interval between successive songs is less than two song lengths. Songs were measured on sonagrams by one of us (PRG). Measurements were analysed by ANOVA and populations were compared by Student–Newman–Keuls (SNK) post-hoc tests.

## RESULTS

### *Phylogeny*

Figure 3 shows the order of branching among the *G. difficilis* populations and congeneric species. All but one of the nodes are supported by bootstrap values at the 50% level. The UPGMA tree, based on the matrix of genetic distances in Table 2, clusters the *G. difficilis* populations on the northern islands of Wolf and Darwin together. Populations on Fernandina and Santiago cluster together, and their closest relatives are on Santa Cruz. The remaining population on Santa Cruz has stronger affinities with other congeneric species.

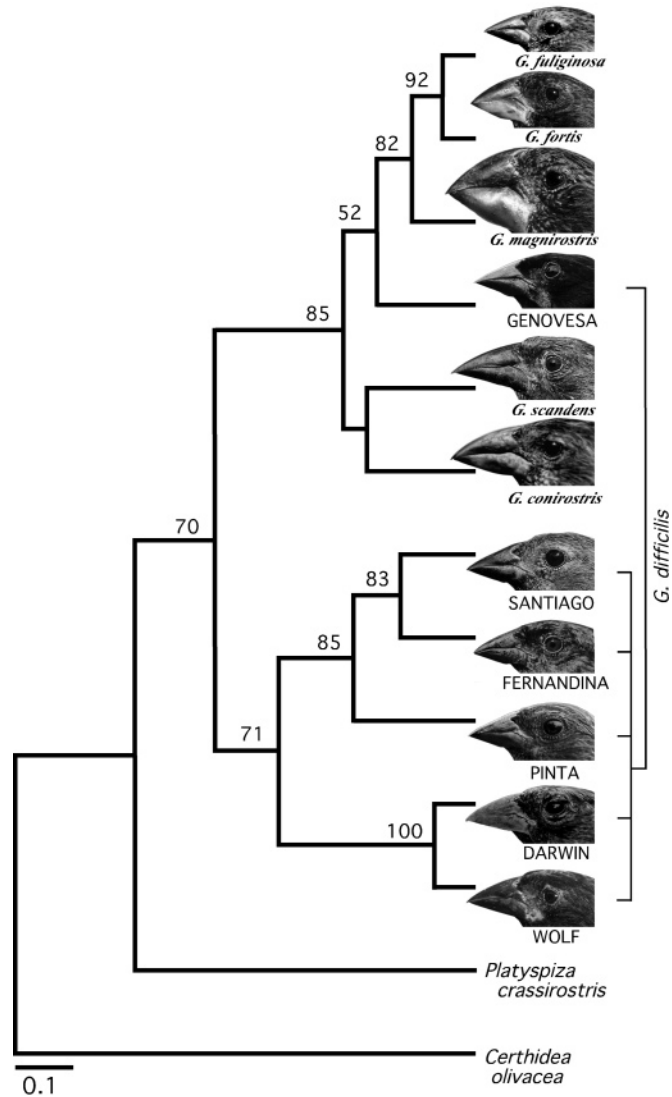


Figure 3. Phylogenetic reconstruction based on UPGMA of Nei's D values from microsatellite DNA data (Table 2). Numbers give percent bootstrap support for nodes from 100 resamplings. One of the warbler finches (*Certhidea fusca*), the Cocos finch (*Pinaroloxias inornata*) and a separate *Camarhynchus/Cactospiza* tree finch branch have been omitted. Populations of the same species on different islands have been combined in the analysis, except for the *G. difficilis* populations (see Petren *et al.*, 1999a for details). Sample sizes of these populations are 30 (Genovesa), 23 (Pinta), 14 (Santiago), 12 (Darwin), 10 (Wolf) and 8 (Fernandina); for the remainder see Petren *et al.* (1999a).

These results lead to rejection of an hypothesis of close relationship between the Genovesa population and the other five populations of *G. difficilis*. In none of the 100 bootstrap resamplings was the Genovesa population of *G. difficilis* placed within or adjacent to the cluster of the other populations of this species to the exclusion of the other *Geospiza* species. An alternative hypothesis of close relationship with *G. fuliginosa* is also rejected. In none of the 100 bootstrap resamplings was the Genovesa



TABLE 2. A genetic distance matrix ( $G_{45}$ , Nei 1972) among Darwin's finches and six island populations of *G. difficilis*

Species/Location	<i>Geospiza</i>						<i>Geospiza difficilis</i> (island populations)						<i>Platyspiza</i>	
	<i>fuliginosa</i>	<i>fortis</i>	<i>magnirostris</i>	<i>scandens</i>	<i>conirostris</i>		Genovesa	Santiago	Wolf	Darwin	Pinta	Fernandina	<i>crassirostris</i>	
<i>G. fuliginosa</i>	—													
<i>G. fortis</i>	0.11	—												
<i>G. magnirostris</i>	0.25	0.19	—											
<i>G. scandens</i>	0.35	0.35	0.47	—										
<i>G. conirostris</i>	0.39	0.36	0.36	0.37	—									
<i>G. difficilis</i>														
Genovesa	0.29	0.31	0.40	0.80	0.52	—								
Santiago	0.63	0.87	1.04	0.98	1.03	1.05	—							
Wolf	0.58	0.83	1.08	0.66	1.05	0.59	1.19	—						
Darwin	0.67	0.80	1.06	0.69	1.08	0.76	1.13	0.14	—					
Pinta	0.69	0.89	1.15	1.04	1.02	0.39	0.94	0.70	0.84	—				
Fernandina	0.47	0.66	0.77	0.75	0.88	0.26	0.81	0.51	0.61	0.45	—			
<i>Platyspiza crassirostris</i>	0.82	1.02	1.26	1.05	1.07	1.23	1.12	1.52	1.38	1.09	1.10	—		
<i>Certhidea olivacea</i>	1.32	1.36	1.41	1.50	1.52	1.62	1.74	1.72	1.35	2.05	1.40	1.73	—	

population of *G. difficilis* placed adjacent to *G. fuliginosa*. The Genovesa population stands alone. A third finding is that *G. scandens* is not closely related to the morphologically somewhat similar populations of *G. difficilis* on Wolf and Darwin according to the results in Figure 3. However, in 12% of the bootstrap resamplings *G. scandens* was grouped with *G. difficilis* on Wolf and Darwin to the exclusion of all other populations.

The arrangement of species does not change when parts of it are reanalyzed with populations of *G. fuliginosa* and *G. scandens* treated separately. For example six populations of *G. fuliginosa* (Daphne Major, Rábida, Pinta, Española, Santiago and Santa Cruz; sample sizes 9–18) cluster together as a monophyletic group, and their position with respect to the Genovesa population of *G. difficilis* is unaltered.

Genetic groupings of *G. difficilis* populations correspond to geographical and ecological groupings (Table 1). There is a high, central, island clade, a northwestern low island clade and a northeastern low island clade. We continue to use the name *G. difficilis* for this third one, despite phylogenetic affinities to other *Geospiza* species, and postpone consideration of its taxonomic status to the Discussion after other evidence has been presented.

### Morphology

Morphologically, the *G. difficilis* taxon is not well defined. The common name of sharp-beaked ground finch derives from a partially straight, not completely curved, culmen, seen in profile in Figure 3. Viewed from above the top of the beak is somewhat flattened where it emerges from the feathers of the forehead. From there to near the tip the beak is relatively uncurved. The pointed feature is least developed or even absent in adults on Wolf and Darwin. It has never been measured directly, although it has been indexed by length to tip from a point along the commissure where beak depth is 4 mm (Schluter & Grant, 1984a). The importance of this feature is that it is shared by the Genovesa population and the populations on the three high central islands, as well as the extinct population on Santa Cruz and possibly the extinct population on Floreana. It is not shown by any of the other *Geospiza* species (*G. fuliginosa*, *G. scandens*, *G. conirostris*, *G. fortis* and *G. magnirostris*).

Morphological differentiation of the populations has involved changes in overall size and in shape. The largest birds are on Santiago (mean 27 g), and the smallest birds are on Genovesa (mean 12 g). Allometric relations vary between each of five dimensions and body mass. The strongest and weakest are illustrated in Figure 4 with two populations of *G. fuliginosa* included with the six populations of *G. difficilis* because of their morphological similarity. The figure illustrates two points. First, considering all eight populations, allometries are much tighter in some cases such as bill width ( $r^2=0.957$ ,  $P<0.01$ ; and tarsus length, not shown,  $r^2=0.929$ ,  $P<0.01$ ), than in others, principally bill length ( $r^2=0.537$ ,  $P>0.1$ ) and the shape factor, bill length at a depth of 4 mm ( $r^2=0.019$ ,  $P>0.1$ ). This shows that bill length and shape have evolved independently of overall body size. Second, points for the Genovesa population are close to an extrapolation of the line of best fit to the other five *G. difficilis* populations in all cases, whereas some values for the *G. fuliginosa* populations are not well predicted by body mass in this way. *G. fuliginosa* differs most from *G. difficilis* in the beak shape factor. The least similar to *G. fuliginosa* is the population on Genovesa. In fact this population has a more acute tip to the beak than all other

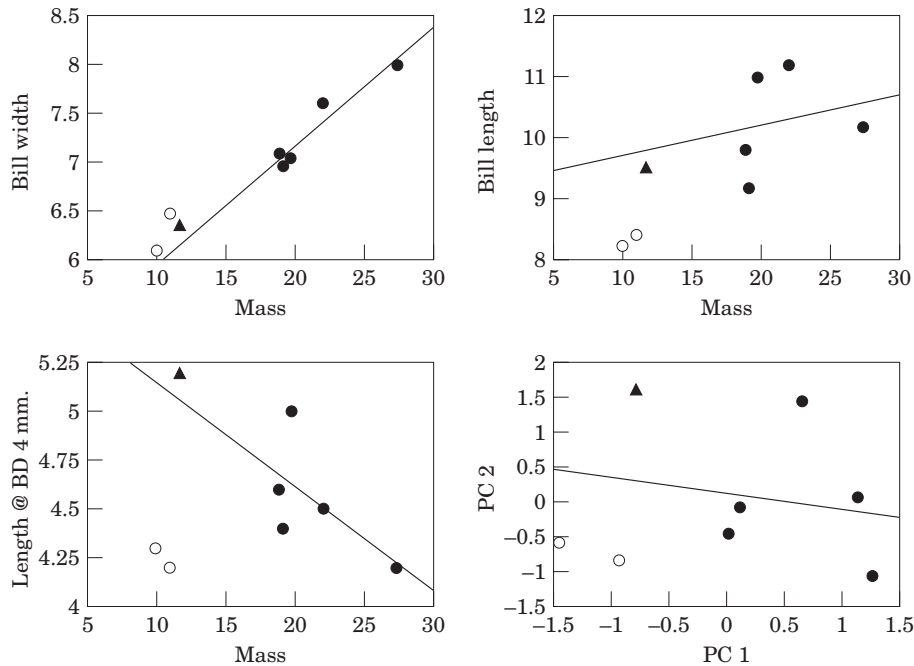


Figure 4. Morphological relationships among six populations of *G. difficilis* (solid symbols) and two populations of *G. fuliginosa* (open symbols). The Genovesa population of *G. difficilis* is identified by a triangle. Least squares lines of best fit to the data from five populations of *G. difficilis*, not including the Genovesa population, are extended to show the degree to which the Genovesa population and *G. fuliginosa* are predicted by extrapolation of that variation. PC 1 and PC 2 are size and shape variables respectively (Table 3); moving away from the origin, size increases on the horizontal axis and beak pointedness increases on the vertical axis. See text for statistical analyses of the full set of eight populations.

populations of *G. difficilis* (SNK tests,  $P < 0.01$  in all comparisons;  $F_{5,182} = 77.985$ ,  $P < 0.001$ ).

Multivariate analyses yield the same conclusion, regardless of whether population means or individual measurements are used in Principal Components analysis, whether weight is replaced by its cube root or deleted from the data set because of its different dimensionality, and whether the beak length at a depth of 4 mm is excluded for the same reason or not. Figure 4 shows only the results of PCA of the population means from the full data set. Table 3 gives details of the analysis. The Genovesa population of *G. difficilis* is the most similar to *G. fuliginosa* in size (PC 1) but the least similar in shape (PC 2). All four differences in size and shape variables between the *G. difficilis* population on Genovesa and the two populations of *G. fuliginosa* are significant by t-tests (two-tailed  $P < 0.001$ , correcting for multiple tests).

Euclidean distance provides a single measure of the difference between any two populations on the two axes. By this measure *G. difficilis* on Pinta (1.30, 1.65) and Fernandina (1.04, 1.48) are closest and hence most similar to *G. fuliginosa*. Distances between *G. difficilis* on Genovesa and *G. fuliginosa* on Pinta (2.45) and Marchena (2.28) are much larger. Therefore, there is no strong morphological reason for grouping the Genovesa population of *G. difficilis* with *G. fuliginosa*, other than on grounds of size similarity.

TABLE 3. Factor loadings for the first two principal components derived from morphological means of six populations of *G. difficilis* and two populations of *G. fuliginosa*

Variables	PC 1	PC 2
Body mass, g	0.416	0.147
Wing length, mm	0.402	-0.230
Tarsus length, mm	0.424	0.007
Bill length, mm	0.379	-0.369
Bill depth, mm	0.410	0.205
Bill width, mm	0.417	0.194
Bill length at 4 mm	-0.004	-0.894
% variance	76.4	19.6

TABLE 4. Samples of recorded songs of *Geospiza difficilis*

Island	Birds	Number of songs (birds; mean/bird; range)	
		Single <sup>1</sup> or repeated <sup>2</sup>	Buzz song <sup>3</sup> or broad band buzz <sup>4</sup>
Darwin	12	0 (0; 0.0; 0-0)	73 (12; 6.1; 4-16) <sup>3</sup>
Wolf	45	261 (45; 5.8; 1-12) <sup>2</sup>	10 (10; 2.2; 0-4) <sup>4</sup>
Genovesa	30	89 (5; 17.6; 2-49) <sup>2</sup>	328 (25; 13.1; 2-84) <sup>3</sup>
Fernandina	6	88 (6; 14.7; 3-34) <sup>1</sup>	20 (3; 6.7; 0-12) <sup>4</sup>
Pinta	46	670 (45; 14.9; 0-46) <sup>1</sup>	255 (40; 6.4; 0-29) <sup>4</sup>
Santiago	16	59 (15; 3.9; 0-8) <sup>1</sup>	29 (12; 2.4; 0-5) <sup>4</sup>

Note. The Wolf sample includes recordings of 10 males from the North Islet. All broad band buzzes were recorded on the main island.

Morphological differences (Euclidean distances) between populations of *G. difficilis* are correlated with genetic distances by a Mantel test ( $r=0.570$ ,  $P=0.0199$ ). Morphological differences are not correlated with geographical distances ( $r=0.191$ ,  $P=0.2510$ ), whereas genetic distances are correlated with geographical distances ( $r=0.591$ ,  $P=0.0040$ ).

### Vocalizations

Loud advertising song, low amplitude broad band buzzes and whistles are heterogeneously distributed among the six island populations. The three central high island populations form one vocalization group, and the three low island populations differ from them and from each other. Samples of recordings are listed in Table 4, a summary of variation in types of vocalizations among the populations is given in Table 5, and the details are illustrated in Figures 5-10.

### Advertising songs

On Santiago, Pinta and Fernandina birds sing a short, moderately complex, loud, tonal, advertising song, usually singly, rarely in pairs or triplets (Figs 5 and 6). Variation among the populations is minor. Advertising songs are formed from five

TABLE 5. Presence (+) or absence (0) of vocalizations of *Geospiza difficilis* in six populations on Galápagos islands

Island	Song		Broad band buzz	Whistle
	Buzzy	Tonal <sup>1</sup>		
Darwin	+	0	0	+
Wolf	0 <sup>2</sup>	R	+ <sup>3</sup>	+ <sup>4</sup>
Genovesa	+	R	0	+
Fernandina	0	S	+	+ <sup>4</sup>
Pinta	0 <sup>2</sup>	S	+	0
Santiago	0	S	+	0

1. Tonal, non-buzzy, songs are generally sung singly (S) or repeatedly (R).
2. Buzzy song recorded by Bowman (1983) rarely on Wolf but not by us or by Ratcliffe (1981), and once on Pinta by us (Fig. 8).
3. Broad band buzz not recorded on the North Islet of Wolf.
4. Follows buzz on main island of Wolf (Fig. 9); independent of song on North Islet of Wolf (Fig. 10), as on Darwin and Genovesa; very short whistle precedes the buzz in recordings of two birds on Fernandina (Fig. 9).

to ten elements and are structurally most complex on Santiago. They are approximately the same length (duration), and all span a broad frequency range beginning at 8-13 kHz and descending.

On the three low islands two different types of advertising song are sung. One is tonal and the other is buzzy (Table 5). The tonal, non-buzzy, song is similar to the song of highland populations but tends to be structurally simpler (Fig. 7). It is sung by all males on Wolf, a minority on Genovesa and by none on Darwin (Tables 4 and 5). High and low island populations differ in these songs in two ways: in repetition and in frequency characteristics. The repetition contrast is illustrated by a comparison of pooled samples of songs from a maximum of 20 males per island. Whereas the percent occurrence of songs sung in multiples of two or three on Santiago (0.0;  $n = 13$  males, 59 songs), Fernandina (20.0;  $n = 5$ , 70) and Pinta (12.4; 20, 322) is low or zero, all songs on Wolf (mode = 4; 20, 133) and Genovesa (mode = 4; 5, 52) are sung in multiples of two to eight. Songs on the latter two islands resemble the songs of *G. fuliginosa*; on Pinta, for example, the range is two to six (mode = 3; 15, 91).

Secondly, frequency minima (1.5–2.5 kHz) are similar on all islands but the maxima and hence ranges differ significantly (F tests,  $P < 0.001$ ). For example maximum frequency varies among the five *G. difficilis* populations and the Pinta population of *G. fuliginosa* ( $F_{5,91} = 50.243$ ,  $P < 0.001$ ), with all three high island populations of *G. difficilis* having higher maxima than the other two and *G. fuliginosa* (SNK tests, all  $P < 0.01$ ). Populations on Wolf and Genovesa do not differ from each other or from *G. fuliginosa* (SNK tests,  $P > 0.05$ ).

The second type of advertising song on low islands has a buzzy quality (Fig. 8). It is sung by most males on Genovesa and all males on Darwin, and is lacking from Wolf and the three high island populations (Table 5). These songs are sung singly, occasionally in pairs and rarely in triplets. *G. fuliginosa* do not sing this type of song.

#### *Broad band buzzes*

On all three high islands a second type of vocalization is produced, a low amplitude broad band buzz (Fig. 9). Frequencies of occurrence are 0.28 on Pinta



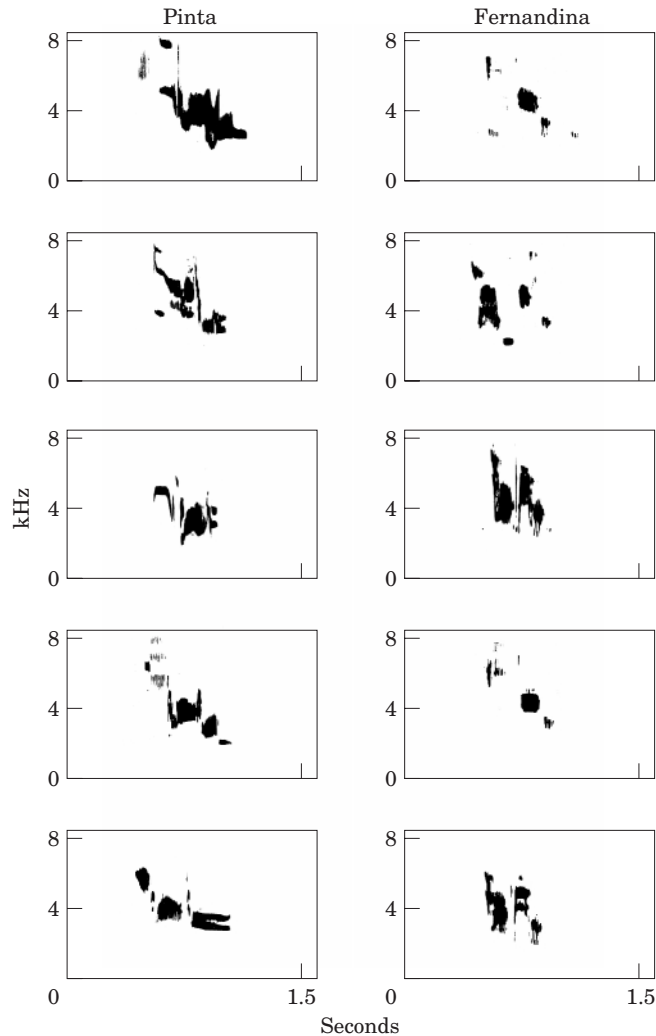


Figure 5. Single songs sung by *G. difficilis* on high elevation islands of Pinta and Fernandina. Five individuals from each populations are shown. See also Figure 6 for the equivalent song sung on Santiago.

( $n = 925$  songs and buzzes recorded), 0.33 on Santiago ( $n = 88$ ) and 0.18 on Fernandina ( $n = 108$ ). Preceding and succeeding notes, and duration and frequency characteristics of the buzz, vary among the island populations (Table 6). The rate of frequency modulation changes shortly after the beginning (Santiago, Fig. 6), towards the end (Pinta, Fig. 9) or is interrupted by a short pulse of sound near the end (Fernandina, Fig. 9). On Pinta and Fernandina but not Santiago the buzz is preceded by a short and loud note that spans a large frequency range and is characterized by a ladder-like appearance on sonagrams (side bands) due to rapid frequency or amplitude modulation (e.g. see Marler, 1967; Stein, 1968; Wiley & Richards, 1982). The buzz is followed by one or more short and loud notes on all three islands. Sound energy of terminal notes is concentrated in a narrow range of constant and low frequencies

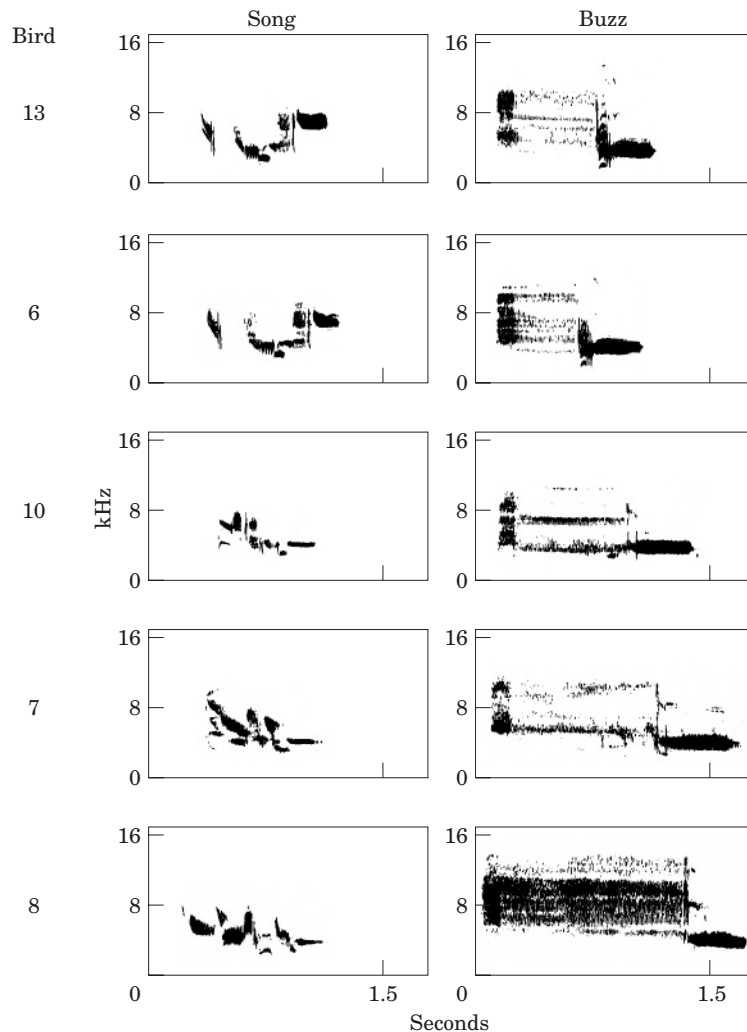


Figure 6. Variation among *Geospiza difficilis* males on Santiago. Songs vary in structure whereas buzzes vary in duration (Table 5).

on Santiago and Pinta but not on Fernandina. The buzz is shorter and the minimum frequency is higher on Santiago than on the other islands, and on Pinta the maximum frequency and the range of frequencies are lower than elsewhere (SNK tests, all  $P < 0.05$ ; see Table 6).

The broad band buzz is also sung on the main island of Wolf, but apparently not on the closely adjacent North Islet, or on Genovesa and Darwin. The absence of the broad band buzz on the North islet of Wolf ( $n = 10$  birds) yet presence on the main island of Wolf less than 100 m away (recorded from 10 of 35 birds) is unexpected. The difference is possibly explained by sampling error (Fisher's Exact  $P = 0.06$ ).

Distributions of broad band buzzes and buzzy songs are complementary: one or the other is present on an island (Table 5). We distinguish the song from the broad

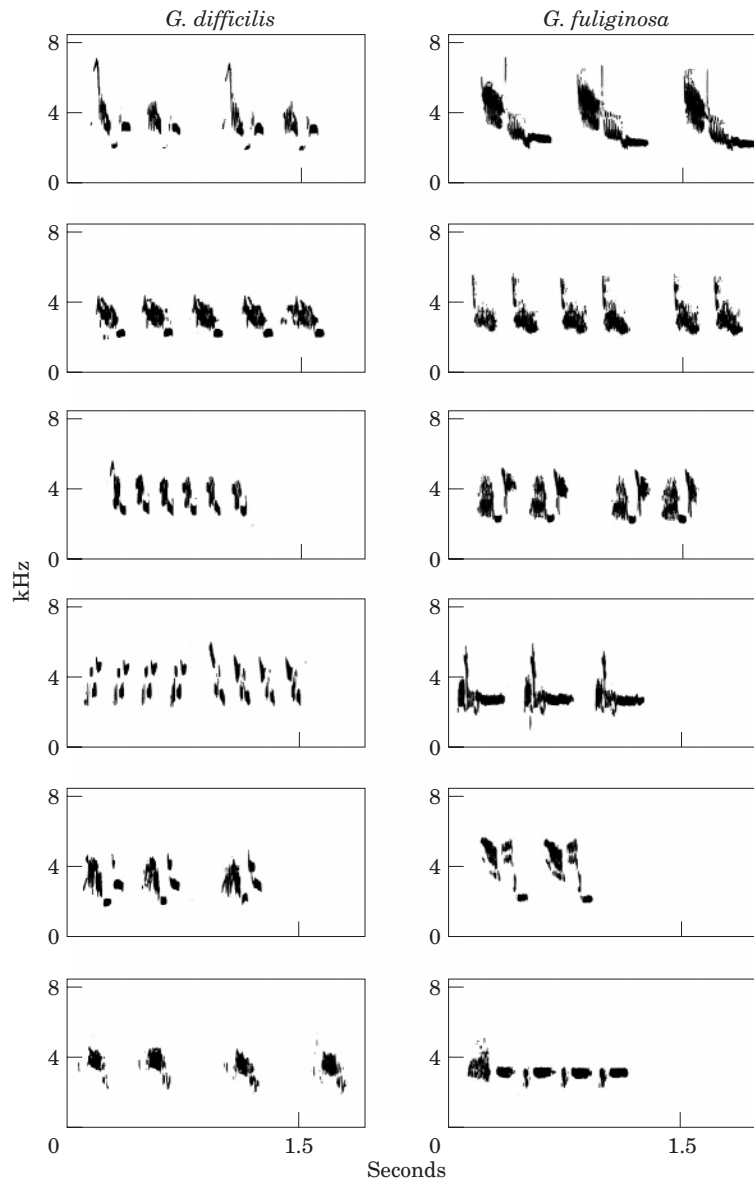


Figure 7. Repeated songs of *G. difficilis* from Wolf (upper five sonagrams) and Genovesa (lowest sonagram), and *G. fuliginosa* from several islands from top to bottom as follows; San Cristóbal, Floreana, Santiago, Pinta, Santa Fe and Fernandina.

band buzz on the basis of a higher amplitude. It is also lower in frequency and shorter. Nevertheless the distinction is blurred on Genovesa where, uniquely, some males sing the buzzy song over a very broad frequency range (Fig. 9), up to a maximum of 14 kHz (see also Bowman, 1983, Fig. 61). In the upper frequency band the amplitude in our recordings is low, as in broad band buzzes on other islands. This vocalization combines features of the advertising song and broad band buzz.

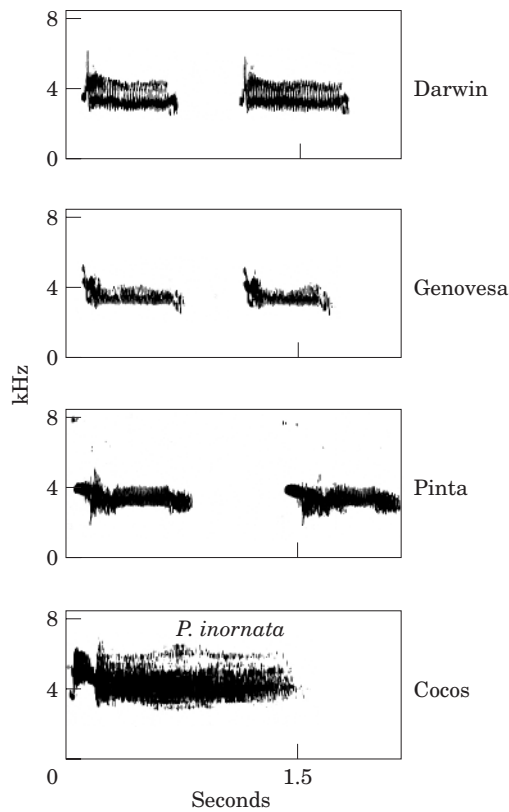


Figure 8. Buzzy songs of *Geospiza difficilis* on Darwin, Genovesa and Pinta, and also *Pinaroloxias inornata* on Cocos island (Cornell cat. no. 86783; recorded by R.I. Bowman). Only one individual was recorded, or heard, singing this song on Pinta. Note the similarity in frequency and temporal characteristics of the Darwin and Genovesa songs. See also Figure 9 for an atypical song on Genovesa.

#### *High-pitched whistles*

Populations of *G. difficilis* display marked variation in the occurrence and characteristics of the whistle (Figs. 9 and 10). Among the high island populations it is present only on Fernandina, as a very short prelude to the broad band buzz (Fig. 9). On the low islands of Genovesa and Darwin a long and very high pitched pure tone whistle is produced independently of other vocalizations (Fig. 10). On the main island of Wolf the whistle is short and immediately follows the broad band buzz (Fig. 9), whereas on the adjacent North Islet it is independent, descends and breaks up in the descent (Fig. 10).

Independent whistles and buzzes appear to be functionally equivalent, not only because they are sung in the same context (Ratcliffe, 1981), but because one or the other is present on all islands, and never both (Table 5).

#### DISCUSSION

The patterns of variation among populations of *G. difficilis* raise two general questions. First, how and why did they become differentiated? Second, what do

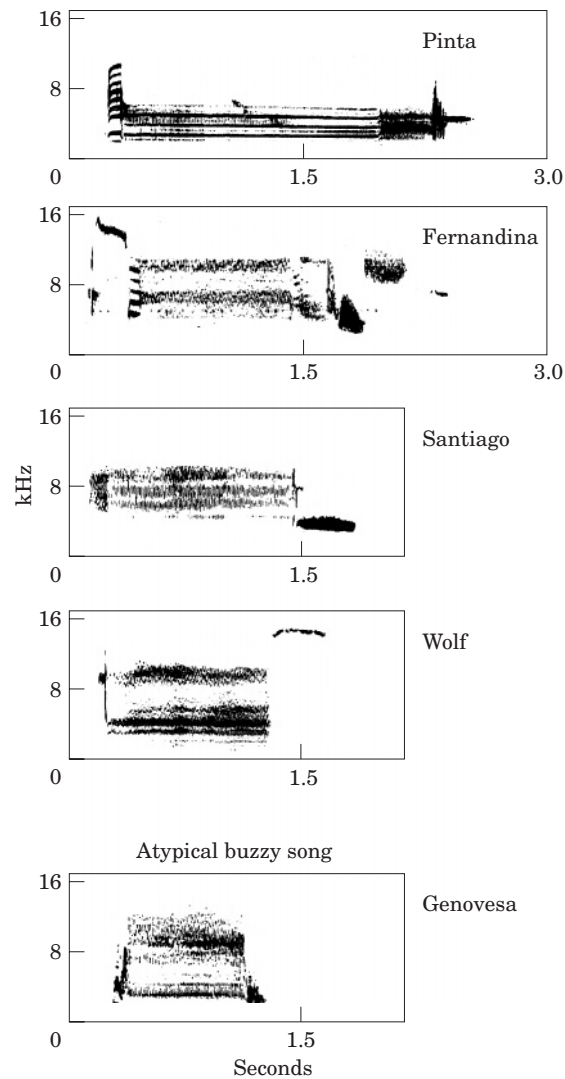


Figure 9. Broad band buzzes of *Geospiza difficilis* on four islands, and a structurally similar song sung on Genovesa (see text). Because the amplitude varies so much during this vocalization on Pinta the illustration is a composite of two sonagrams; the buzz is much lower in amplitude than the rest. Note the high-pitched whistle immediately following the buzz on Wolf, and low-pitched terminal whistles on Santiago and Pinta.

their differences imply about speciation? Before addressing them we first discuss the evolution of the taxonomically enigmatic population on Genovesa.

#### *Evolutionary history of the population on Genovesa*

Results of the phylogenetic analyses confirm the grouping of five of the six populations of the taxon we currently refer to as *G. difficilis*. However the Genovesa



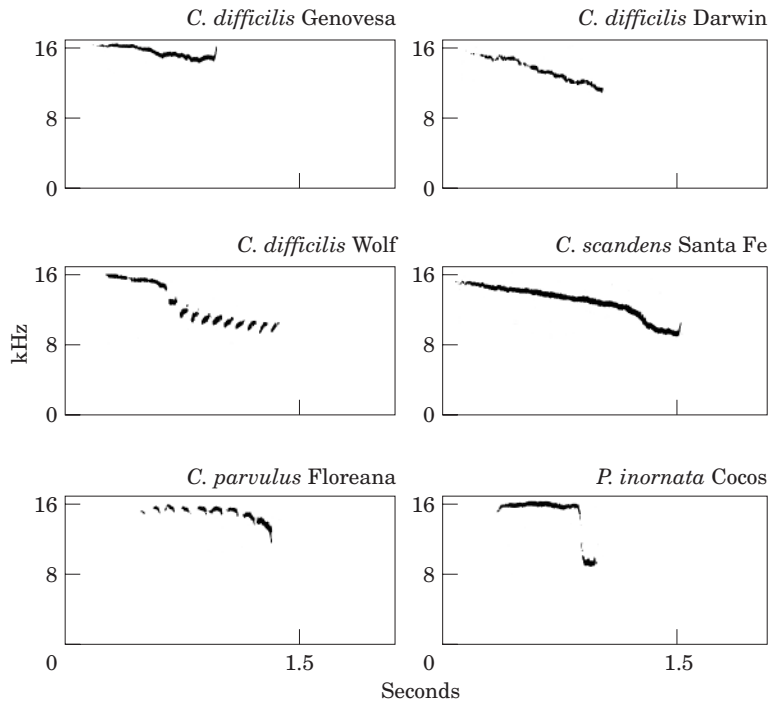


Figure 10. High-pitched whistles. Populations of *Geospiza difficilis* on Darwin and Genovesa sing long whistles, as do all populations of the other five *Geospiza* species (illustrated here with *G. scandens*), *Platyspiza crassirostris* and *Pinaroloxias inornata* (Cornell cat. no. 86783). A unique 'broken' pattern in the whistles on the North Islet of Wolf is similar to the broken pattern of the three species of *Camarhynchus*, illustrated here by *C. parvulus*.

TABLE 6. Characteristics of broad band buzzes of *G. difficilis*

Island	N	Duration (s)		Minimum		Frequency (kHz)		Range	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Santiago	12	0.84	27.90	3.11	0.58	12.15	1.28	9.04	1.33
Pinta	8	1.53	37.82	1.44	0.49	7.81	3.25	6.37	1.05
Fernandina	3	1.35	12.49	2.00	—	12.43	0.75	10.43	0.75
Wolf	6	1.12	18.74	2.00	—	13.00	1.90	11.00	1.90

Note. Populations differ in duration ( $F_{3,26}=11.49$ ), minimum frequency ( $F_{3,26}=22.98$ ), maximum frequency ( $F_{3,26}=9.79$ ) and frequency range ( $F_{3,26}=7.14$ );  $P<0.001$  for all tests.

population is genetically more similar to other species of the genus, and does not form a monophyletic group with the other five populations of *G. difficilis*. Is it therefore irrelevant to a discussion of the evolution of *G. difficilis*? A simple answer would be yes, but the situation is made complex by the lack of concordance between phenotypic and genetic variation.

The morphological analyses confirm previous studies in showing that the Genovesa population resembles other populations of *G. difficilis* in shape and *G. fuliginosa* in size (Schluter & Grant, 1984a; Vagvolgyi & Vagvolgyi, 1989, 1991; Schluter *et al.*,

1991). Analyses of vocalizations show that individuals on Genovesa have (a) features in common with other populations of *G. difficilis* but not shared by other *Geospiza* species (buzzy song and high frequencies), and (b) features shared both conspecifically and heterospecifically (whistles and repeated songs). Thus trait variation (morphology and vocalizations) does not closely match genetic variation (cf. Lynch & Baker, 1986; Baker *et al.*, 1990), nor do morphological and vocal traits covary completely. Lack of concordance presents a problem when attempting to reconstruct evolutionary history and assess phylogenetic affinities (Patterson, 1987). Difficulties are greatest when times between divergence events are short, differentiation is extensive and variable, and hybridization (reticulation) occurs (Patton & Smith, 1994), all of which are applicable to Darwin's finches.

Lack (1945) originally proposed that the Genovesa population was derived from ancestral *G. fuliginosa* (but see also Lack, 1947). This has no genetic support, rather the genetic data show that evolution of the Genovesa population preceded the origin of *G. fuliginosa*. According to the phylogenetic reconstruction in Figure 3, the proto-*Geospiza* lineage split into two, one leading to the five populations of *G. difficilis*, the other leading to the modern Genovesa population of *G. difficilis*. This second lineage gave rise to two derived lineages, the first to the long-billed species *G. scandens* and *G. conirostris*, and the second to the blunt-billed granivores, *G. magnirostris*, *G. fortis* and *G. fuliginosa*.

Retention of ancestral traits in the Genovesa population is indicated by three features. First, the sharp beak profile is similar to the beak profile of *G. difficilis* populations on the three high islands, and to no other species. Second, the buzzy song is almost identical to the song of *G. difficilis* on Darwin, and different from all other Galápagos species. Third, rufous wing bars (females) and rufous tips to the white under-tail coverts (males) occur rarely in Genovesa birds and in *G. difficilis* on Pinta, and more commonly in the other populations of *G. difficilis*. They are not present in *G. fuliginosa* on any of the islands, nor any congeners except for some *G. magnirostris* on Darwin and *G. conirostris* on Genovesa (Rothschild & Hartert, 1899; Snodgrass & Heller, 1904; Lack, 1945, 1947; Schluter *et al.*, 1991). They are shared to some degree by the older species, *Certhidea*, *Platyspiza crassirostris* and *Pinaroloxias inornata* (Lack, 1945, 1947).

These similarities with *G. difficilis* could be due alternatively to convergence or, in the case of learned vocalizations, to copying from another species. Ecological data reject an hypothesis of convergence in beak traits; the Genovesa environment and its food supply differs strongly from the highland habitats occupied by *G. difficilis* on Santiago, Pinta and Fernandina (Schluter & Grant, 1982, 1984a). Convergence in plumage traits is possible but not explained. The buzzy song could have been acquired by copying from another population of *G. difficilis* or, with strong modification, from *Certhidea*. This explanation has two disadvantages: it requires another population of *G. difficilis* that is no longer present on Genovesa, and it requires an increase in the frequency of heterotypic song from just above zero to more than 80%. While this is possible by cultural drift or frequency dependent sexual selection it is not supported by direct evidence from studies of *G. fortis* and *G. scandens* on Daphne (Grant & Grant, 1996ab, 1997a,b). Like beak shape and plumage, vocalization similarities are more plausibly explained by shared ancestry.

Similarities between populations in genetic and phenotypic traits can also arise from introgressive hybridization. Another possibility to consider is that the Genovesa population of *G. difficilis* has been genetically affected by hybridization with other

congeneric species which evolved later. The effect on phylogenetic reconstruction would be to place the population closer to other species than is correct by actual genealogy. Hybridization between members of this population and *G. conirostris* is known to occur, but is very rare (Grant & Grant, 1989) and is unlikely to produce the topology in Figure 3. Hybridization with the only other congener on Genovesa, *G. magnirostris*, is not known (Grant & Grant, 1989).

Hybridization in the past with immigrants of another species is possible, for example *G. fuliginosa* from the neighbouring islands of Marchena, Santiago and Santa Cruz. Although plausible the past hybridization argument suffers from the disadvantage that for hybridization to have had its postulated genetic effects on the Genovesa population, immigration would have had to occur massively or repeatedly at a time of low population density on Genovesa, or a resident, hybridizing, population of *G. fuliginosa* or other species has become extinct. This discussion illustrates the general problem of assessing the respective contributions of shared ancestry and introgressive hybridization to the genetic similarities of taxa that are known to hybridize (Avice, 1989; Smith, 1992; Jackman & Wake, 1994; Clarke *et al.*, 1997).

To summarize, the Genovesa population was derived from *G. difficilis*, as revealed by beak shape, plumage and vocalization traits, but is genetically well differentiated from it as a result of early divergence and possibly in part as a result of hybridization. Regardless of the name applied to it (see below), the Genovesa population is not irrelevant to the discussion of *G. difficilis* evolution.

#### *Morphological evolution in allopatry*

Reconstruction of character evolution is helped by establishing ancestor–descendant relationships from an estimate of their phylogeny (e.g. Funk, 1996; Givnish & Sytsma, 1997). Only one such relationship can be inferred within the *G. difficilis* clade (Fig. 3); the Pinta lineage is ancestral to the populations on Santiago and Fernandina.

Even without ancestor–descendant relationships it is possible to make inferences about the process of differentiation by comparing related populations. The number of possible directional changes is reduced when populations that are geographically close are also genetically similar, as is the case here. Using the comparative approach, though without information on genetic similarity, Schluter and Grant (1982, 1984a) tested three alternative explanations for the morphological differentiation of *G. difficilis* populations. They concluded that differentiation occurred as a result of adaptation to local food supplies that differed among islands, most conspicuously between high and low altitude habitats.

The evidence is as follows. On Wolf and Darwin the relatively long beaks of *G. difficilis* are associated with exploiting *Opuntia* flowers for pollen and nectar, feeding on blood from developing feathers of sea birds (*Sula* species), and cracking their eggs and eating the contents (Schluter & Grant, 1984a). On Genovesa the small body size and small pointed beaks of *G. difficilis* are correlated with a dry season diet of nectar from the small flowers of *Waltheria ovata* and small seeds. *G. fuliginosa* uses a similar beak to feed on similar foods on other islands. Diet and density of *G. difficilis* on Genovesa are well predicted by the same attributes of *G. fuliginosa* at the lowland sites on Pinta and Marchena, which are similar to Genovesa in vegetation, substrate

characteristics and food supply, and not well predicted by diet and density of *G. difficilis* at highland sites on Pinta, Santiago and Fernandina (Schluter & Grant, 1982). Thus the niche for a sharp-beaked finch is present on many islands, and it is defined by different factors on some of those islands.

Although both *G. difficilis* and *G. fuliginosa* feed on *Waltheria* flowers, they do so in different ways that illustrate the mechanical differences between beaks of slightly different shape. *G. difficilis* uses the whole beak to probe a flower whereas *G. fuliginosa* uses just the lower mandible and tongue. It is not known if they differ in efficiency of nectar extraction (Schluter & Grant, 1982). One possible adaptive reason for the small body size of *G. difficilis* on Genovesa is that it enhances perching on the slender stems of *Waltheria* bushes to reach these flowers (Schluter & Grant, 1984a).

#### *Differentiation of vocalizations in allopatry*

Different habitats, with different compositions and structures of plant communities, differ in their physical properties of sound transmission (see Morton, 1975, 1982; Wiley & Richards, 1982; Bowman, 1983; Wiley, 1991; Endler, 1992). A correspondence is expected between the vocal characteristics of singing birds and the sound-transmitting properties of their environment, because there should be an advantage to minimizing vocal signal attenuation and degradation. When males efficiently transmit their songs over a long distance they presumably gain a sexually selected advantage from enhanced mating success, and a naturally selected advantage from reduced interference from competitors (see also Price, 1998).

Sound transmission experiments yield a simple high–low rule for efficient long distance transmission; sing on high perches at low frequencies (Martin & Marler, 1977; Martin *et al.*, 1977). Three other factors have been suggested to influence vocal signal characteristics; body size, the songs of other species and ambient noise (Bowman, 1979, 1983; Brenowitz, 1982; Wiley & Richards, 1982; Ryan & Brenowitz, 1985; Martens & Geduldig, 1990; Klump, 1996; Badyaev & Leaf, 1997). Responses to the songs of other species are not predictable because they depend on the nature of those songs, whereas maximum frequency systematically declines with increasing body size, and increases with ambient, generally low frequency, noise.

Male *G. difficilis* conform to the high perch–low frequency rule. On the high islands they also sing at high frequencies which enhances their locatability in the relatively dense vegetation of the *Zanthoxylum* forest (e.g. see Wiley & Richards, 1982). Body size is not a factor in the frequency variation, but ambient noise may be. On the low islands loud ambient noise at 1–4 kHz is generated by nesting boobies (*Sula* species) and terns (*Sterna fuscata*). Under such conditions increasing the signal to noise ratio can be accomplished by producing alerting notes or by repetition of notes or phrases in a song (Wiley & Richards, 1982). Male *G. difficilis* produce both. Repetitive notes with a simple structure are produced solely on the low islands of Wolf and Genovesa. Loud introductory (and terminal) notes of the broad band buzz and the buzzy song can be interpreted as serving the alerting function. These are produced on the three low islands, as well as on Pinta and Fernandina.

In short distance communication, birds often use high frequencies (e.g. Titus, 1998) and wide spectrum sounds resulting in maximum locatability in forested habitats or with high ambient noise (Wiley & Richards, 1982). *G. difficilis* use sound energy in the high frequency range of 8–16 kHz in two contrasting ways: diffusely

in wide spectrum buzzes in the forested habitat of the high islands, and concentratedly in a narrow spectrum whistle in the relatively open habitats of the low islands.

Thus some of the differences in vocalizations between high and low island populations can be explained as the result of selection in different sound transmitting environments. Not all differences can be explained by current environmental variation, and two discrepancies among the low islands in particular are conspicuous. The first involves the broad band buzz. This is sung on Wolf, an island of open habitat, but not apparently on the adjacent and floristically similar North Islet, and not on Darwin and Genovesa. Wolf is most likely to have been colonized by birds from Pinta that sang the broad band buzz. Its presence on Wolf may simply reflect its origin from another island, and the lack of a disadvantage rather than any advantage on Wolf.

The second discrepancy is between Wolf and Darwin. Geographically close, topographically and floristically similar, these islands support populations that are morphologically and genetically similar. Vocalizations should be similar too, yet they differ strongly: the repeated song and broad band buzz are sung on Wolf and the buzzy song and high-pitched whistle are sung on Darwin. Two hypotheses for this pattern involve chance. One is loss of the buzzy songs and most of the whistle from the Wolf population through cultural drift at low population numbers, and likewise loss of the repeated song and the broad band buzz from the Darwin population, perhaps in the founding of that population (e.g. see Baker & Jenkins, 1987; Baker *et al.*, 1990; Lynch & Baker, 1994; Baker, 1996). An alternative is colonization by birds with different vocalizations. The two islands may have been colonized by genetically similar but vocally different immigrants from elsewhere in the archipelago, and it was a matter of chance which vocal types arrived at each island.

The hypothesis of loss through cultural drift assumes a broad range of vocalizations as the ancestral condition. Evidence for this is provided by the *Certhidea* species, under the assumption that their current vocalizations were possessed by the immediate ancestor of *G. difficilis*. The assumption is reasonable because vocalizations of the combined populations of *G. difficilis* are basically similar to those of the two *Certhidea* species. All populations of *Certhidea* that have been studied (Bowman, 1983, this study) display the continuous though relatively short whistle, invariably associated with (preceding) advertising songs, structurally simple and repeated or complex versions of those songs, and broad band buzzes (Fig. 11). Vocalizations of *G. difficilis* were therefore inherited from their *Certhidea* progenitors and underwent modification and, in some cases, reduction during population differentiation. For example the simple repeated songs evolved from the condition shown by *C. olivacea* through lengthening of the notes and reduction in the high frequencies used (see Fig. 7). The whistle became independent of the advertising song in Genovesa and Darwin populations of *G. difficilis*, and was lost on Pinta and Santiago.

#### *Implications for speciation*

In the evolutionary history of *G. difficilis*, differentiation of populations was strongly influenced by habitat differences among the islands. These differences may have become accentuated through time as a result of fluctuations in climate and the different effects of those fluctuations on the habitats of islands of different size and



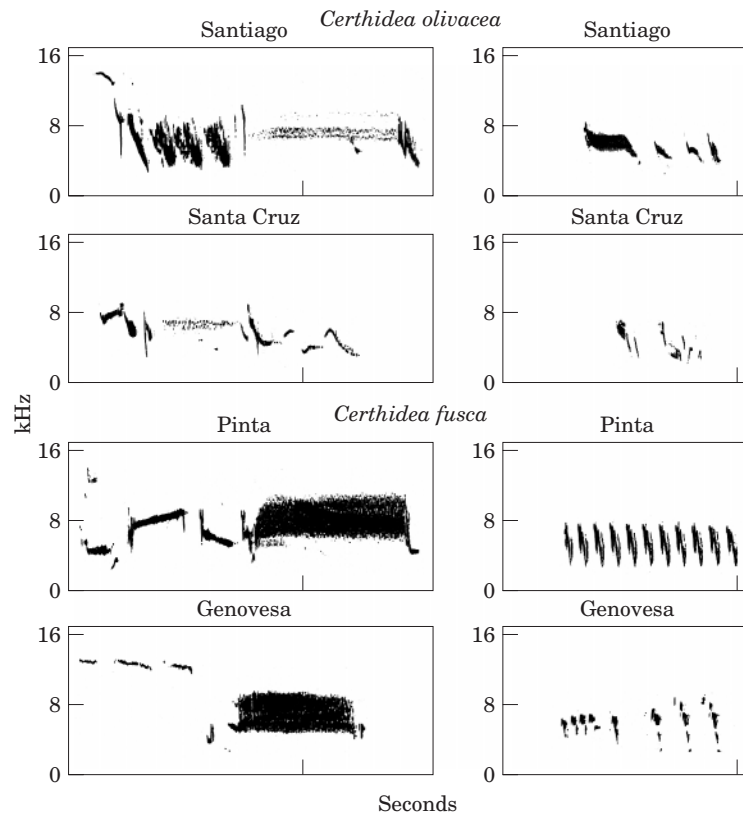


Figure 11. Vocalizations of the two *Certhidea* species comprise whistles, buzzes and repeated notes. The two recordings from Pinta are from the same bird.

elevation. Indeed colonization may have been facilitated by a more widespread distribution of *Zanthoxylum* forest under cooler conditions than now prevail. Ecologically the populations differentiated by adapting in beak morphology to different, and possibly changing, food supplies. Since beak size and shape also function in conspecific mate recognition and choice, the potential for reproductive isolation from sister and parental taxa arose as a correlated effect of natural selection on resource-exploiting traits (Grant, 1986). This conforms to a standard explanation for the origin of pre-mating isolation; it arises as a byproduct of adaptive changes in allopatry without being adaptive itself (Dobzhansky, 1937; Mayr, 1942; Rice & Hostert, 1993; Butlin & Ritchie, 1994; Carson, 1997).

However, this explanation is incomplete. Vocal characteristics also differentiated, partly as a result of natural and sexual selection acting independently of beak size change, and partly as a result of chance. The chief implication for speciation is that pre-mating isolation, at least in birds, is not simply the incidental consequence of adaptive change in other traits in allopatry. For species that use more than one modality for communicating in courtship, the scope for pre-mating isolating factors to arise in allopatry and be subject to sexual selection is large (e.g. see Price, 1998).

Given this scope, has differentiation of *G. difficilis* progressed to the point at which their potential to interbreed is substantially reduced through the evolution of barriers

TABLE 7. Discrimination indices for pairs of populations of *G. difficilis*. Euclidean distances (D) between population means of multivariate morphology are shown above the diagonal, and vocalization differences on a scale of 0 (no difference) to 2 (complete difference) are shown below the diagonal. See text for further details

	Genovesa	Darwin	Wolf	Santiago	Pinta	Fernandina
Genovesa	x	2.02	1.82	1.62	0.32	0.43
Darwin	0	x	0.28	0.52	2.01	1.70
Wolf	0.5	1	x	0.54	1.85	1.54
Santiago	2	2	1	x	1.56	1.26
Pinta	2	2	1	0	x	0.31
Fernandina	2	2	1	0	0	x

to mating? This is, in essence, a question of applying the biological species concept to allopatric populations. It can be accomplished only with difficulty (e.g. see McKittrick & Zink, 1988; Zink & McKittrick, 1995) because it is necessarily speculative. Usually, in the absence of a natural test of interbreeding, morphological differences between sister taxa in sympatry are used as a guide in assessing the status of allopatric populations (Mayr, 1942, 1963; Peterson, 1998). Some authors have pointed out that behavioral differences may be a better guide than morphology (Butlin & Ritchie, 1994), especially differences in vocalizations (e.g. Isler *et al.*, 1998, 1999; Peterson, 1998). Experimental studies of Darwin's finches have been conducted with both morphological (Ratcliffe & Grant, 1983a,b) and behavioral (vocal) cues (Ratcliffe & Grant, 1985), and these, combined with breeding studies of populations (Grant, 1986; Grant & Grant, 1989), enable us to address the speciation question with quantitative data.

Experiments with museum specimens of females showed that male discrimination between own type and an 'immigrant' from an allopatric population is a function of the morphological difference between them, as indexed by  $z$ -scores. Results fell into two discrete classes: discrimination was weak ( $z < 0.4$ ) or strong ( $z > 0.6$ ). These results can be used to establish a morphological criterion for discrimination that can be applied to the *G. difficilis* populations, as follows.

First, Principal Components analysis is performed on published mean values (Grant *et al.*, 1985) of wing, tarsus, bill length, depth and width for the populations studied in morphological discrimination tests (Ratcliffe & Grant, 1983b) and for all *G. difficilis* populations. Second, Euclidean distances (D) between pairs of populations on the two Principal Components axes are calculated, and the strongly and weakly discriminated populations are then compared. Differences between weakly discriminated populations are in the range 0.61 to 0.90, much lower than differences between strongly discriminated pairs (1.55 to 2.77). Differences between *G. difficilis* populations fall into two morphological groups (Table 7). One group comprises Genovesa, Pinta and Fernandina populations (D = 0.31–0.43), the other group comprises populations on Wolf, Darwin and Santiago (D = 0.28–0.54), and between members of different groups the lowest D values are 1.26 and 1.54 (Fernandina compared with Santiago and Wolf) and the highest is 2.02 (Genovesa and Darwin). Thus members of one morphological group would presumably discriminate against members of the other.

An attempt is made in this table to represent differences in vocalizations as well, by considering advertising song as one vocal trait and the buzz/whistle as a second

trait, and giving them equal weight. Whistles and buzzes were not used in the experimental tests, therefore we have been forced to make an assumption about their role in mate choice. Equal weighting is that assumption. A value of 0 is given for shared identical traits and 2 is given for completely different traits. This categorization of differences yields a different grouping, one that matches more closely the ecological grouping (Table 1). The low island populations form one group and the high island populations form another group, although there is some ambiguity in the placement of the Wolf population.

The speciation question can now be addressed by setting up criteria specifically designed for these comparisons. We suggest joint membership in both morphological and vocal groups would potentially lead to interbreeding and fusion into a single panmictic population. Joint membership in neither group confers reproductive isolation, despite a possibly low level of interbreeding. Joint membership in one but not the other leaves taxonomic status open. Thus to be considered a separate species under the biological species concept a population would have to be a member of a different morphological and vocal group from other populations.

Application of these criteria shows that there is a continuum of compatibility among *G. difficilis* populations. Darwin and Wolf populations comprise one compatible pair and Pinta and Fernandina populations comprise another. The incompatible pairs are (1) Darwin and Pinta or Fernandina populations, and (2) Genovesa and Santiago populations. All other pairwise combinations of populations are intermediate. None of the populations stands out as potentially isolated from all or even a majority of the rest by the way we have constructed the table of discrimination values and applied criteria of compatibility. In particular the population on Genovesa is not excluded unequivocally from a group comprising mutually compatible populations.

The results imply that the probability of interbreeding in a hypothetical context of secondary contact (sympatry) will vary among different combinations of populations. This implication is supported by observations of interbreeding among a different set of species on Daphne Major (Grant & Grant, 1992, 1997a,b, 1998).

To sum up, for morphological and behavioral (vocal) reasons, members of some populations would probably not breed with members of other populations. Populations are well advanced down three paths of speciation. One, on Genovesa, has passed the more easily reached point of deserving species recognition under the phylogenetic species concept (see below). None of the populations is likely to be completely isolated reproductively from all others by pre-mating barriers, hence none deserves species recognition under the biological species concept, paraphyletic difficulties notwithstanding. These conclusions about potential reproductive interactions are tentative and need to be tested by additional playback experiments using allopatric recordings of songs, whistles and buzzes (cf. Gill & Murray, 1972; Becker, 1977; Ratcliffe & Grant, 1985; Payne, 1986; Salomon, 1989; Baker & Baker, 1990; Lynch & Baker, 1990; Baker, 1991, 1994). The outcome of a hypothetical secondary contact, and the possible completion of the speciation process, would depend on other ecological and epigamic factors; on ecological resources and competitors and the potential for coexistence (Schluter & Grant, 1984b), and on other species that are similar in morphological and vocal traits, as these are known to modify the discriminations (Ratcliffe & Grant, 1983b).

*Taxonomic status of the population on Genovesa*

From a phylogenetic perspective the best taxonomic treatment of the Genovesa population is to consider it a distinct species, using for it the name it was first given: *G. acutirostris* (Ridgway, 1894). It is diagnosably different from all members of the *G. difficilis* assemblage in body size, it is diagnosably different from the *G. fuliginosa* assemblage in bill shape, and it is not part of a monophyletic assemblage of either. It remains part of the superspecies *G. difficilis* (Lack, 1945), which appears to be paraphyletic, a characteristic of the early stages of radiations (Avise, 1994; Patton & Smith, 1994; Avise & Wollenberg, 1997) and of re-radiations from just one of the lineages.

Under the biological species concept, however, assignment to *G. acutirostris* is not well supported, as discussed above. If the Santiago population was the only other population referable to *G. difficilis* the issue would be clear; the Genovesa population would be almost certainly reproductively isolated from it, and the two would be considered different species. However, between these two extremes lie intermediate populations. By the same criteria applied to the Santiago and Genovesa populations, one population, on Darwin, would be reproductively isolated from two others, those on Pinta and Fernandina. Yet neither the Genovesa population on the one hand nor the Santiago population on the other are clearly isolated morphologically and behaviorally from the populations on Darwin, Pinta or Fernandina. Recognizing these difficulties we follow the traditional practice of considering the Genovesa population to be a well-differentiated population of the *G. difficilis* species.

In reaching this taxonomic decision we have chosen uncertain biological criteria over certain genetic criteria because they are more compatible with the general theory of adaptive radiation. According to this theory a new population is founded by dispersers from the original population, and the process of speciation is initiated by evolutionary change in the new environment, for example a new island (Mayr, 1942, 1963; Lack, 1945, 1947; Grant, 1986; Grant & Grant, 1996b, 1997c). Speciation then continues in allopatry and secondary sympatry, and during this time evolutionary change in the original population is relatively minor. Speciation is thus conceived of as a process of budding (Mayr, 1974), followed by expansion and evolution of the bud.

In contrast speciation is thought of as a process of splitting in phylogenetic reconstruction based on cladistic principles. Here an ancestral species splits into two, losing its identity in the process. Splitting can be justified in terms of mutual genetic divergence of presumably neutral genetic markers used in the reconstruction of taxonomic affinities. However, for relatively recent radiations such as Darwin's finches these genetic differences are irrelevant to speciation, or at best less relevant, than are morphological and behavioral traits used in courtship and mate choice. Neutral genetic markers do not mark mate choice syndromes closely enough to be useful in their absence, nor can they even be considered markers of genetic differences affecting post-zygotic fitness because these arise only after considerable time in the divergence of bird species (Grant & Grant, 1992, 1997c). In the short-term there is no morphological or behavioral clock analogous to a molecular clock, whereas over time spans of several millions of years clock-like patterns may be evident (Coyne & Orr, 1998).

## ACKNOWLEDGEMENTS

We thank the Galápagos National Parks Service and the Charles Darwin Research Station for permission and support in carrying out the fieldwork on Galápagos, and the Costa Rican National Parks Service for similar support for our work on Cocos island. L.M. Ratcliffe recorded most of the vocalizations on Pinta. D. Day helped us with recordings on Santa Cruz, Wolf and Darwin. T.W. Sherry supplied us with a tape-recording of finches on Cocos island, and M. Fischer of the Cornell laboratory of natural sounds provided us with sonagrams which we used in Figures 8 and 10. S. Nowicki and S. Peters allowed us to use the Sonograph to prepare all other sonagrams. We thank D. Schluter for morphological measurements, K.T. Grant, M. Hau and M. Wikelski for field assistance, and T.D. Price and two referees for comments on the manuscript. The research was supported by grants from NSERC (Canada) and NSF (USA), most recently DEB 93-06753 and 96-15383.

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