

### A. Estimates of effective population sizes

We estimated effective population sizes based on the levels of nucleotide diversity in populations and an estimate of mutation rate of  $2.04 \times 10^{-9}$  per base per year from a comparison between a Darwin's finch and zebra finch<sup>45</sup> (Extended Data Table 2). These estimates of effective population sizes ( $N_e$ ) are up to two orders of magnitude higher than census estimates for the populations studied in detail on Genovesa<sup>60</sup> and Daphne Major<sup>48</sup>. It is very likely that the relatively high effective population sizes and nucleotide diversities are largely explained by gene flow within species between islands as well as between species; the census estimates for populations on other, much larger, islands are substantially higher than those on Genovesa and Daphne Major.

### B. Taxonomic revision

Traditional taxonomy is based on phenotypes<sup>3,18</sup>. Strongly differentiated allopatric populations have been problematic because the interbreeding test of species rank cannot be performed<sup>61-63</sup>. Two species of Darwin's finches in particular have given taxonomic difficulties, *Geospiza difficilis* and *G. conirostris*<sup>6,18,64</sup>. Our genomic data (Fig. 1b) do not support the traditional treatment of either of these taxa and we therefore propose a revised taxonomy for these species.

**Sharp-beaked ground finch (*G. difficilis*).** There are clearly three groups (Fig. 1b), and a revised taxonomy should reflect this. We recommend recognition of three species as originally classified:

*G. acutirostris* Ridgway; found on Genovesa.

*G. difficilis* Sharpe; found on Pinta, Fernandina and Santiago.

*G. septentrionalis* Rothschild and Hartert; found on Wolf and Darwin.

Members of the *difficilis* taxon as recognized above are closely related to each other in all three genetic measures (autosomes, mitochondria and Z-chromosomes), and distinct from the other two groups (Fig. 1b; Extended Data Fig. 5). Morphologically they differ from *septentrionalis* in having a straight culmen (hence "sharp-beaked") and they differ in song<sup>56</sup>. They are much larger in body size than *acutirostris* (Extended Data Table 1). The two populations of *septentrionalis* are more closely related to each other genetically than to any other population of ground finches, and they differ from both *difficilis* and *acutirostris* in having a curved culmen.

*G. acutirostris* is sister to *fortis* (autosomes) or *fuliginosa* (Z). Morphologically, especially in the size and shape of the beak, they resemble Pinta *difficilis* except in size (Extended Data Table 1, Extended Data Fig. 4a), and that is why they were originally classified as *difficilis*<sup>18</sup>. They resemble *fuliginosa* more closely in body size (Extended Data Fig. 4a) but not in beak proportions or in their straight culmen, and they do not resemble the much larger *fortis*. In song they resemble *septentrionalis*<sup>56</sup>. There is no resemblance to *fuliginosa* or *fortis* in song. Heterogeneous genetic affinities suggest mixed ancestry. We consider them to be neither *difficilis* nor *fuliginosa*, and merit recognition instead as a distinctive taxon.

Extinct breeding populations of *difficilis* were present on Santa Cruz Island, certainly, on Floreana, probably, and on Isabela, possibly. A few specimens exist in museums<sup>65</sup>. Morphologically, genetically and in habitat they appear to be part of the *difficilis* taxon<sup>18,65</sup>.

**Large cactus finch (*G. conirostris*).** The Genovesa and Española populations cannot be maintained as the same species. Each is more similar genetically to another species than to each other: *scandens* for the Genovesa population and *magnirostris* for the Española population. This applies to autosomal affinities as well as to morphology<sup>4</sup> and song<sup>60,66</sup>. Song playback experiments on Genovesa showed that *G. conirostris* responded only weakly to the songs of Española *G. conirostris*<sup>67</sup>. The reciprocal experiments on Española were not performed.

In view of their genetic and morphological distinctiveness we propose that each population should be recognized as a separate species. We recommend retaining the name *G. conirostris* Ridgway for the populations on Española and its satellite Gardner as originally described<sup>68</sup>, and resurrecting the name *G. propinqua* Ridgway for the Genovesa population.

Morphological distinctiveness is illustrated in Extended Data Fig. 4b. Average body size varies gradually among species, whereas beak shape variation exhibits a step function, and the steps are spaced apart equally. This is significant because beak shape is an important trait in species recognition<sup>4</sup>. On this axis *conirostris* on Española (and Gardner) and *propinqua* on Genovesa lie between and are clearly separated from a *scandens* cluster and a *magnirostris* cluster. *G. propinqua* on Genovesa and *G. conirostris* differ by 0.17 beak shape units, *G. conirostris* differs from the most similar population of *G. magnirostris* by the same amount (0.17), and *G. propinqua* differs from the most similar *G. scandens* population by almost the same (0.20). Differences between neighbouring populations of *G. scandens* on the same axis are much smaller, 0.06 beak shape units at maximum, and the same is true for *G. magnirostris* (0.06).

In spite of the distinctiveness of these species there is inevitable uncertainty over whether allopatric populations would interbreed if they came into contact. Responses of birds to playback of song recorded on another island help to reduce the uncertainty by simulating the encounter of members of allopatric populations. The specific distinctness of *conirostris* on Española is supported by the results of playback experiments. Male *conirostris* did not respond at all to playback of *magnirostris* songs recorded on Santa Cruz Island<sup>67</sup>, despite structural similarity of the songs<sup>66</sup>. Evidence of the specific distinctness of *propinqua* on Genovesa is mixed. Males responded as strongly to one type of *scandens* song recorded on Daphne Major Island as they did to their own song. However, *scandens* on Plaza Sur Island clearly discriminated between own song and the song of *propinqua*. Furthermore male *scandens* discriminated strongly against *propinqua* when tested with a pair of museum specimen models in female plumage, one of *propinqua* and one of local *scandens*: the reciprocal experiment on Genovesa was not completed<sup>69</sup>. Evidence for discrimination suggests that *propinqua* and *scandens* would rarely interbreed.

**Implications.** Raising the number of species from 15 to 18 shortens the average time to speciation<sup>1,70</sup> from  $1.00 \times 10^5$  years to  $0.83 \times 10^5$  years, and elevates the species accumulation curve (Extended Data Fig. 4c). The indicated accelerating upward trend towards the present is likely to be a characteristic of young radiations in which introgressive hybridization is

prevalent<sup>5</sup>, but may also reflect increasing opportunities for speciation associated with recent glacial-interglacial cycles of environmental change<sup>7,8</sup>.

### C. Dating the nodes in the phylogeny

We dated the nodes in the phylogeny using levels of genomic divergence between species assuming a mutation rate of  $2.04 \times 10^{-9}$  per base per year<sup>45</sup>, after correcting the divergence between taxa for time to coalescence in the ancestral population using estimates of extant nucleotide diversity<sup>49</sup>. The estimates based on nuclear DNA (Fig. 2a) are similar to those obtained from *cytochrome b* sequences using a fossil-calibrated divergence rate of 2.1% per base per million year for bird mtDNA<sup>46</sup> (Extended Data Fig. 5a). For example, the emergence of the Cocos finch is dated as 529,000 and 454,000 years ago by the whole genome and mtDNA data, respectively. However, the deepest split in the radiation, between warbler finches and other finches, is estimated as early as 1.4 million years ago based on mtDNA while nuclear data indicated a split 0.9 million years ago. Moreover, the position of *C. fusca* in the mtDNA tree (Extended Data Fig. 5a) is in conflict with the species tree (Fig. 1b) because of gene flow, as described in the main text.

**D. mtDNA data provide evidence for interspecies gene flow.** The mtDNA phylogeny supported gene flow in the distant past between an ancestor of *C. fusca* and a common ancestor of all non-warbler finches, because *C. fusca* showed significantly higher mtDNA sequence identity to all non-warbler finches than to the other warbler finch, *C. olivacea* (Extended Data Fig. 5a). The mtDNA tree also revealed further evidence of gene flow among the tree and ground finches. One *G. conirostris* individual on Genovesa carried an mtDNA sequence that clustered with mtDNA sequences from tree finches (*Camarhynchus* group), implying successful interspecies hybridization despite clustering with other *G. conirostris* individuals in the autosomal genome phylogeny. Thus, this individual does not represent a recent hybridization, but descends from an introgressed matriline from a tree finch. There are no resident tree finches on Genovesa, but rare immigrants may occur<sup>60</sup>. ABBA-BABA analysis also provided strong evidence for introgression between tree finches and *G. conirostris* on Genovesa ( $P=7 \times 10^{-234}$ ; SI Table 2). The mtDNA data are also consistent with extensive gene flow among ground finches, unlike the earlier-formed species, since their mtDNA sequences did not form species-specific clusters (Extended Data Fig. 5a).

### E. Demographic history

To elucidate and display the demographic history of Darwin's finches we used the pairwise sequentially Markovian coalescent (PSMC) model, which infers changes in effective population size over evolutionary time from a single genome sequence<sup>21</sup>. First, we compared the demographic trajectories from populations of all Darwin's finches excluding *G. difficilis* (Extended Data Fig. 6c), since these populations are not monophyletic as shown in the main text. The observed demographic patterns were highly consistent among individuals of the same species and among closely related species. The analysis revealed that the ground finches have maintained larger effective population sizes than other species of Darwin's finches. Second, a comparison of the six populations of *G. difficilis* revealed that the lowland populations on Genovesa, Wolf and Darwin, which all have a mixed ancestry involving

hybridization with other ground finches, have had higher long-term effective population sizes than the *G. difficilis* populations occupying the highlands of Pinta, Santiago and Fernandina (Extended Data Fig. 6d). Effective population sizes have apparently fluctuated over time. Noteworthy is the approximate correspondence between peaks in effective size for some of the ground finches and glacial maxima at ~120,000 and ~20,000 years ago when greatest exposure of land occurred<sup>7,8</sup>. Highland populations of *G. difficilis*, occupying restricted habitat at high elevation, do not show these pronounced peaks.

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