

27. K. Andersen *et al.*, *Mol. Ecol.* 10.1111/j.1365-2011.05261.x (2011).
28. A. Paus, G. Velle, J. Berge, *Quat. Sci. Rev.* **30**, 1780 (2011).
29. T. Alm, *Boreas* **22**, 171 (1993).
30. T. O. Vorren, K.-D. Vorren, T. Alm, S. Gulliksen, R. Løvlie, *Boreas* **17**, 41 (1988).
31. K. D. Vorren, *Boreas* **7**, 19 (1978).
32. T. Alm, H. H. Birks, *Nord. J. Bot.* **11**, 465 (1991).
33. P. Taberlet *et al.*, *Nucleic Acids Res.* **35**, e14 (2007).
34. T. Preuten *et al.*, *Plant J.* **64**, 948 (2010).
35. U. Rauwolf, H. Golczyk, S. Greiner, R. G. Herrmann, *Mol. Genet. Genomics* **283**, 35 (2010).
36. J. Haile *et al.*, *Mol. Biol. Evol.* **24**, 982 (2007).
37. L. Parducci, Y. Suyama, M. Lascoux, K. D. Bennett, *Mol. Ecol.* **14**, 2873 (2005).
38. T. O. Vorren, L. Plassen, *Boreas* **31**, 97 (2002).
39. J. Mangerud, in *Quaternary Glaciations—Extent and Chronology, Part 1—Europe* (Elsevier, Amsterdam, 2004), pp. 271–294.

Acknowledgments: We thank T. Giesecke and M. Lascoux for helpful comments; J. Chen, T. Geburek, and C. Sperisen for providing modern samples; and L. Yuan for initial spruce genotyping. We also thank T. Vorren and K.-D. Vorren for providing Andøya core samples with lithostratigraphy and radiocarbon dates. This study was supported by the Swedish Research Council (grant 2007-4490 to L.P.) and the Carl Trygger's Foundation (grant 08:303 to L.P.); KAKENHI (22658046); a Royal Society–Wolfson Merit Award to K.D.B.; the Danish National Research Foundation; the Norwegian Forest and Landscape Institute, University of Tromsø; and the Tromsø University Museum, the Roald Amundsen Centre for Arctic Research, and the European Commission under the Sixth Framework 378 Programme (EcoChange project, contract no. FP6-036866). L.P., T.J., M.M.T., and E.E. should be considered joint first authors. I.G.A. and E.W. should be considered joint senior authors. The authors declare no competing financial interests. P.T. is coinventor of patents related to the *g/h* primers and the use of the P6 loop of the chloroplast *trnL*

(UAA) intron for plant identification using degraded template DNA. These patents only restrict commercial applications and have no impact on the use of this locus by academic researchers. The *trnL* DNA sequences can be found at the European Nucleotide Archive with the study accession no. ERP001153. The mtDNA sequences can be found at the European Molecular Biology Laboratory database with the accession no. HE652882-HE653002.

Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6072/1083/DC1
Materials and Methods

SOM Text

Figs. S1 to S5

Tables S1 to S5

References (40–92)

1 November 2011; accepted 26 January 2012

10.1126/science.1216043

Founder Effects Persist Despite Adaptive Differentiation: A Field Experiment with Lizards

Jason J. Kolbe,^{1*†} Manuel Leal,² Thomas W. Schoener,³ David A. Spiller,³ Jonathan B. Losos¹

The extent to which random processes such as founder events contribute to evolutionary divergence is a long-standing controversy in evolutionary biology. To determine the respective contributions of founder effects and natural selection, we conducted an experiment in which brown anole (*Anolis sagrei*) lizard populations were established on seven small islands in the Bahamas, from male-female pairs randomly drawn from the same large-island source. These founding events generated significant among-island genetic and morphological differences that persisted throughout the course of the experiment despite all populations adapting in the predicted direction—shorter hindlimbs—in response to the narrower vegetation on the small islands. Thus, using a replicated experiment in nature, we showed that both founder effects and natural selection jointly determine trait values in these populations.

Island populations are renowned for their extent of divergence from each other and from mainland source populations (1, 2). Mayr (3, 4) argued that these differences are often triggered by random sampling when island populations are founded by a few colonizing individuals. The resultant founder effects—changes in the genetic and phenotypic composition of a population due to founding by a small number of individuals—have been proposed as an important cause of evolutionary divergence and even speciation for the past half-century (3–6). However, an alternative explanation is that island environments differ from each other and from the source locality, and these ecological differences result in divergent natural selection (7–9). The evolutionary significance of founder effects also has been

questioned because their imprint may be short-lived if populations perish because of a lack of genetic variation or because of demographic stochasticity, or if natural selection overwhelms their effects (10, 11). Data from nature are lacking because founder events are rarely observed [but see (12)], and thus their effects must be inferred post hoc; yet laboratory studies indicate that even in the presence of natural selection, genetic drift induced by founder effects or population bottlenecks can contribute to patterns of phenotypic divergence [e.g., (13, 14)]. We report an experimental study of founder effects in a natural system of a Caribbean lizard, demonstrating that morphological divergence caused by the founder effect persists even as populations adapt to their new environments.

Some have argued that marked phenotypic change from a single founder event is very unlikely (11) and that genetic drift in general plays little part in morphological evolution (15). Moreover, others note that evidence from recent introductions suggests that adaptation is a more common cause of trait evolution than drift (16–18). Yet most concede that documenting these random sampling events in nature is exceedingly difficult (11, 15, 19–21). Without knowledge of founder

attributes and repeated sampling of colonized islands, the relative contributions of founder effects, population bottlenecks, natural selection, and gene flow to evolutionary divergence are impossible to disentangle (19–21).

We capitalized on the extensive knowledge of how *Anolis* lizards adapt to their environment, combined with the opportunity to use small islands in the Bahamas, recently cleared of lizards by a hurricane, as experimental units in a natural setting. Our focus was on limb length and the extent to which lizard populations would adapt to the novel environments on these small and scrubby islands. Extensive comparative and experimental research supports an adaptive explanation for the positive relationship between hindlimb length and perch diameter (i.e., the width of the cross section of the substrate that a lizard perches on, such as a branch or trunk) that is found among populations of anoles. Functional studies show a clear biomechanical basis: Lizards with relatively longer hindlimbs run faster on broad substrates, whereas lizards with shorter limbs for a given body size move more adeptly on narrow surfaces. Such performance probably aids in capturing prey, defending territories, and escaping from predators. Indeed, field studies in this system show that hindlimb length is under selection, favoring longer hindlimbs to run faster when exposed to terrestrial predators and shorter hindlimbs after lizards become arboreal and shift to narrower perches [reviewed in (22)].

To assess the relative importance of founder effects versus natural selection, we introduced lizards to replicate islands to which we predicted they were not well adapted because of differences in structural habitat from their source, thereby simulating founding events and altering the selective regime for limb length. Specifically, founding propagules were established on small experimental islands, all of which are sparsely vegetated and covered primarily with short, narrow-diameter vegetation as compared to the more forested habitat of the lizards' source area on a nearby larger island (Fig. 1 and figs. S1 and S2). Our prediction is that if natural selection is the dominant force, then we would expect all populations to evolve shorter hindlimbs as they adapt to using narrower

¹Department of Organismic and Evolutionary Biology and Museum of Comparative Zoology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA. ²Department of Biology, Duke University, Durham, NC 27708, USA. ³Section of Evolution and Ecology and Center for Population Biology, One Shields Avenue, University of California Davis, Davis, CA 95616, USA.

*To whom correspondence should be addressed. E-mail: jkolbe@gmail.com

†Present address: Department of Biological Sciences, University of Rhode Island, Kingston, RI 02881, USA.

substrates; moreover, vegetation differences among experimental founder islands would be expected to produce a relationship between hindlimb length and perch diameter [reviewed in (22)]. Conversely, if founder effects are dominant, then we would expect no general trend in limb length evolution, with some populations increasing in limb length and others decreasing with respect to the source population, and limb variation being unrelated to vegetation differences among islands.

In May 2005, we randomly selected one male and one female brown anole from lizards collected on a nearby larger island (Iron Cay, Bahamas, vegetated area >150,000 m²) to found populations on seven small islands (vegetated area 35 to 175 m²). The storm surge from Hurricane Frances in September 2004 submerged these small islands near Great Abaco, extirpating their lizard populations (23). We took morphological measurements and tissue samples for later genetic analysis from these founders. Over the next 4 years, we characterized the environment and repeatedly sampled lizards from the source population (Iron Cay), from the seven experimental founder islands, and from 12 nearby reference islands (vegetated area 74 to 324 m²) having *A. sagrei* and a composition of spider, insect, and plant species similar to that of the experimental islands (24). All lizard populations increased in size for the first 2 years (averaging a 13-fold increase) and fluctuated in size thereafter (Fig. 2).

A founder effect was immediately apparent, conforming to theoretical expectations and previous empirical studies of genetic variation (25–27). In 2006, 1 year after introduction, multilocus genotypes from six microsatellite loci revealed an average decrease of 46% in allelic diversity and 23% in heterozygosity on experimental founder islands as compared to their source. Furthermore, allele frequencies differed significantly among all pairs of populations, including the source on Iron Cay (Stouffer's *z* method of combining probabilities from exact *G* tests of six microsatellite loci; all *P* < 0.05; table S1). Founder populations were dispersed around the mean value of the source population (Iron Cay) and varied fivefold in their amount of divergence from it; moreover, among-islands genotype frequencies in subsequent years were correlated with the genotypes of the two founding individuals [correlation coefficient (*r*) = 0.80 to 0.97 on principal coordinates (PCo) axes 1 to 3 for 2006, 2007, and 2009, all *P* < 0.05; Fig. 3]. Islands differed significantly from each other in genotype frequencies (analyses of variance for PCo 1 to 3 separately, *F*_{6,248} = 16.5 to 41.7, all *P* < 0.0001), whereas differences both across years and among islands across years were not significant. In all but one case (the island N3–Iron Cay comparison), pairwise fixation index (*F*_{ST}) values indicated significant genetic differentiation between islands (table S2), although *F*_{ST} values should be interpreted cautiously because of the likelihood of nonequilibrium conditions in these recently established populations. Moreover, genetic diversity was sustained

after the founding event; only 1 locus out of 42 on the seven islands (six loci per island) showed a net decrease in the number of alleles from founding in 2005 to 2009. These results support the persistence of the initial founder effect without subsequent genetic drift through time.

A founder effect also was evident for hindlimb length, with experimental founder islands differing significantly in 2006 (*P* = 0.0015; table S3 and

Fig. 4, inset). As with the genetic data, hindlimb values for island populations were dispersed around the mean value of the source population on Iron Cay (fig. S3). Furthermore, there was no relationship between perch diameter and relative hindlimb length among islands in 2006 (*r*² = 0.15, *P* = 0.39), supporting the interpretation that differences in hindlimb length among islands resulted from the founder event rather than adaptive divergence.

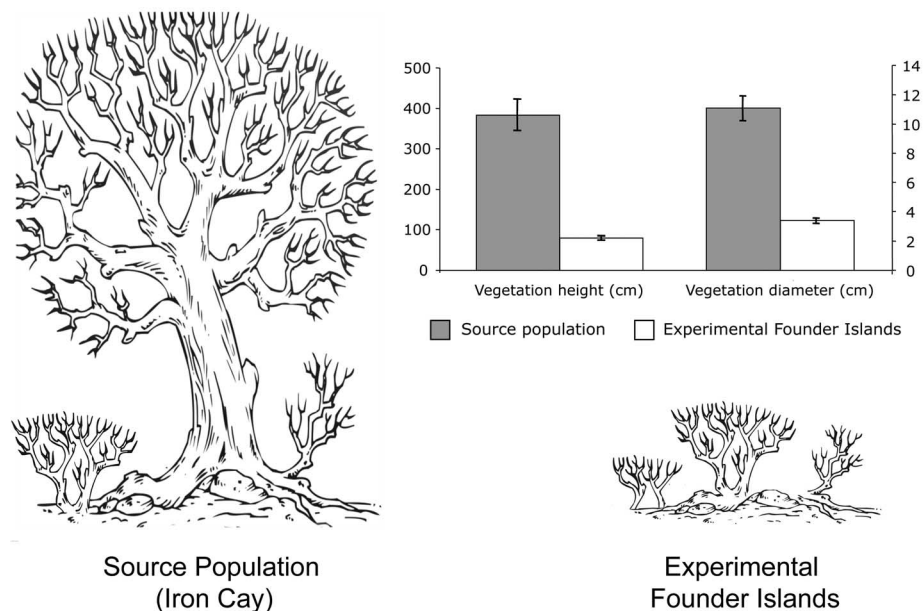


Fig. 1. Vegetation differences between the source population and those of the experimental founder islands. The schematic cartoon shows the change in the vegetation profile for lizards introduced from the source population on the more forested Iron Cay to the sparsely vegetated experimental founder islands. The vegetation illustrated is scaled to the mean values of vegetation height for Iron Cay and the pooled experimental founder islands, respectively. Bar graphs show the difference (mean ± SE) in available vegetation height and diameter from systematic transects on each island. We predicted that the change in vegetation profile would result in lizards using narrower perches on experimental founder islands, which would alter the selective regime to favor shorter hindlimbs.

Fig. 2. Population size estimates for experimental founder islands. Surveys were conducted in May of each year from 2005 to 2009 [see (29) for details]. Population size showed a positive relationship with vegetated area of each island in 2006 (*r*² = 0.53, *P* = 0.06) and 2007 (*r*² = 0.74, *P* = 0.01), suggesting an effect of habitat area on population growth over the first 2 years. Population size estimates from November 2006, which included hatchlings produced that year, ranged from 21.4 to 60.2 (not shown in this figure). In all but one case, the November 2006 size estimates greatly exceeded the population size in May 2007, suggesting high mortality rates. Rapid population expansion such as found here may produce conditions favorable to evolution by natural selection (16, 30) and may also curtail the loss of genetic variation after the initial founder effect.

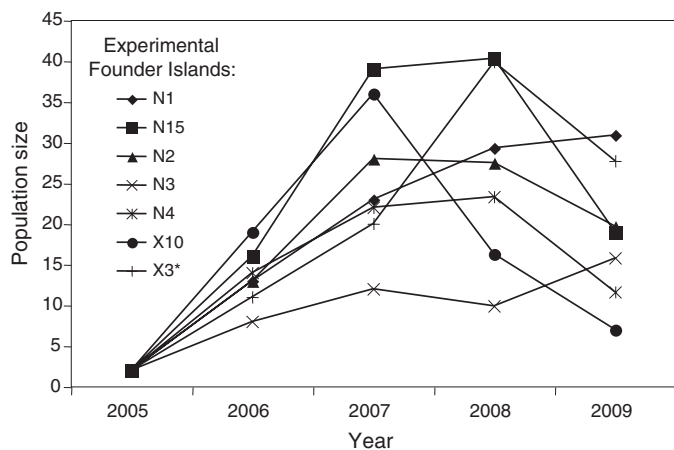


Fig. 3. Multilocus genotypic variation for the source population and those of the experimental founder islands. We conducted a PCo analysis of six microsatellite loci (mean \pm SE of PCo values). Numbers within each symbol correspond to different sampling years: I, 2005 (i.e., the founding pair on each experimental founder island and these same founder individuals pooled for the source population estimate on Iron Cay); II, 2006 (no Iron Cay estimate); III, 2007; and IV, 2009. The percentage of variation explained for each PCo axis is in parentheses. Stored sperm use was detected in 6% of offspring through 2006, resulting in offspring fathered by a male other than the one introduced onto the island. However, all other individuals sampled during this time had multilocus genotypes consistent with being progeny of the founding pair (or founding female in the case of stored sperm use), and not until 2007 did we detect unambiguous immigrants.

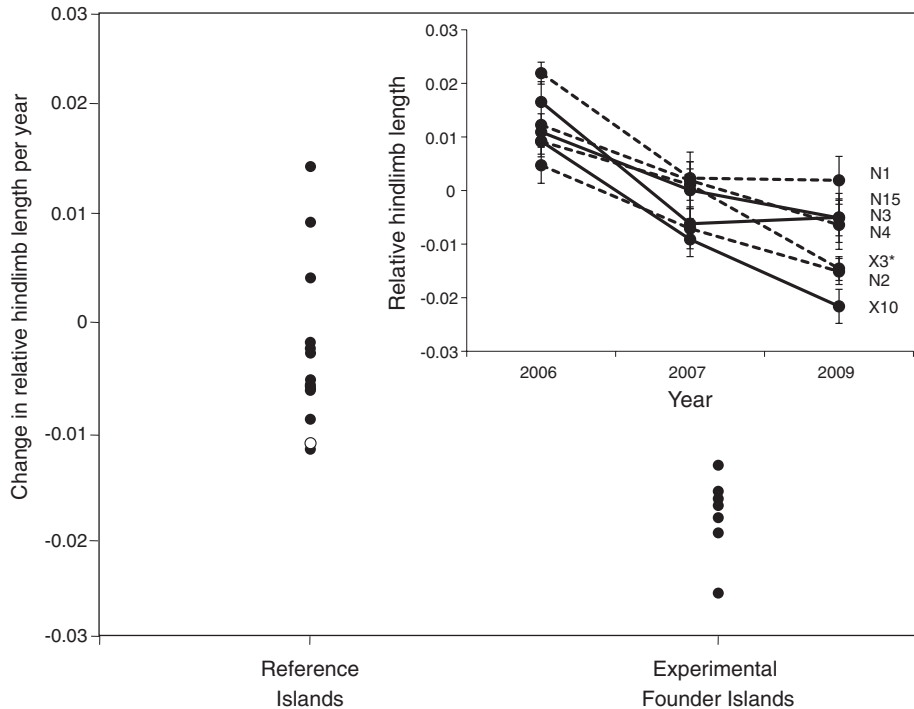
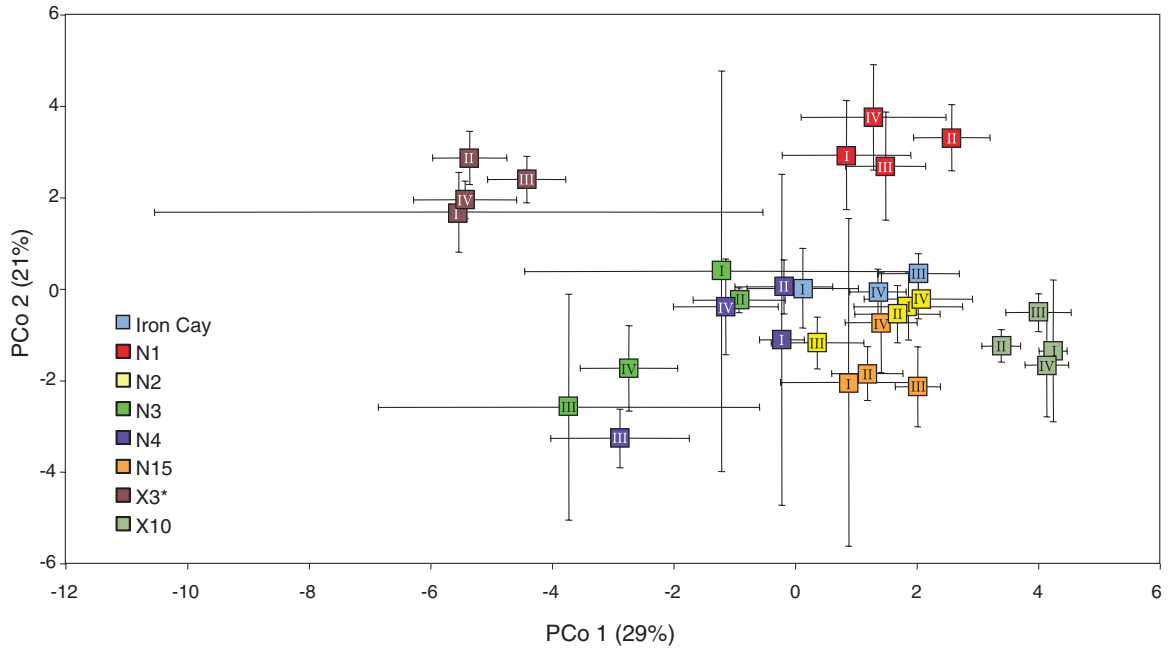


Fig. 4. Change in hindlimb length on reference and experimental founder islands. Change in mean relative hindlimb length [calculated as the residuals of the regression of log-hindlimb length on log-body length (i.e., snout-vent length), separately for each sex] per year for populations on experimental founder and reference islands, including the source population on Iron Cay (open circle). The inset in the upper right shows how the mean (\pm SE) relative hindlimb length decreases on the experimental founder islands in each year. Islands N1, N3, and X3* are shown with dotted lines for clarity. Immigration does not alter the results for hindlimb differentiation, which are virtually identical when putative immigrants are removed from analyses. Although the source population (Iron Cay) decreased in hindlimb length, all lizards on experimental founder islands exhibited even greater decreases than those on Iron Cay and on all other reference islands, with the mean rate of hindlimb change on reference islands not differing from zero.

We used systematic transects to quantify the available structural habitat (i.e., vegetation height and diameter). The vegetation profile of the source population on Iron Cay averaged substantially taller and broader vegetation (mean vegetation height = 384 cm and mean vegetation diameter = 11.1 cm) as compared to the experimental founder islands, which have much shorter and narrower vegetation (mean vegetation height ranged from 62 to 107 cm and mean vegetation diameter ranged from 1.6 to 5.4 cm) (Fig. 1 and figs. S1 and S2). In turn, the mean perch diameter used by lizards on all experimental founder islands (3.2 cm) was smaller than for the source population (4.6 cm).

As predicted by the adaptive relationship between hindlimb length and substrate diameter (22), relative hindlimb length decreased greatly over the course of 3 years on all seven experimental founder islands ($P < 0.0001$; table S4 and Fig. 4, inset), with a decrease of 6.5% for males and 4.0% for females (calculated at the median body size for each sex; table S5 and fig. S4). The magnitude of this decrease in hindlimb length was so great that mean values in 2006 are nearly non-overlapping with 2009 values (Fig. 4, inset). We monitored nearby reference islands for the same length of time, and the decrease on experimental founder islands was five times greater than on the reference islands [mixed effects analysis of covariance (ANCOVA) test for an island type (reference versus experimental founder islands)-by-year interaction: $P < 0.0001$; table S6 and Fig. 4]. The rates of decrease on all seven founder islands were greater than the rates for all reference islands, including the source population on Iron Cay (Fig. 4), and the mean rate for the reference

islands did not differ significantly from zero ($t = 1.55$, $df = 12$, $P = 0.15$), whereas the mean rate for the experimental founder islands did ($t = 11.53$, $df = 6$, $P < 0.0001$).

Despite this substantial adaptive response, the mark of the founder event persisted, as illustrated by the significant difference among islands (ANCOVA: $P < 0.0001$; table S4) and the minimal change in the order of mean hindlimb length values among experimental founder islands from 2006 to 2009 (year-by-island interaction: $P = 0.70$; table S4 and Fig. 4, inset). Furthermore, multiple regression confirms the persistence of the founder effect by showing that mean relative hindlimb lengths among islands in 2006 had a positive relationship with values in 2009 ($P = 0.046$), and it demonstrates that no relationship existed between hindlimb lengths in 2009 and the diameter of either available vegetation ($P = 0.17$) or perches ($P = 0.86$), indicating that adaptation to differing environments cannot explain variation among islands in hindlimb length.

Hindlimb growth can be affected by environment during ontogeny in *A. sagrei* (28), but such phenotypic plasticity is unlikely to explain the large decreases in hindlimb length detected on the experimental founder islands from 2006 to 2009. If plasticity were important, we would have expected to see an immediate difference in hindlimb length between the founders and their first-generation descendants due to the latter developing in the new, narrow-vegetation environment, but the range in population means in 2006 overlapped completely with that of the male founders. Moreover, perch diameter did not change over time on experimental founder islands (year: $P = 0.10$, and year-by-island interaction: $P = 0.13$), even as hindlimb length progressively decreased, showing that the change in hindlimb length cannot be attributed to a change in a possible stimulus for plasticity. Finally, the magnitude of hindlimb divergence is three to five times greater than that produced by plasticity in *A. sagrei* raised on only narrow or broad surfaces in laboratory experiments (28) (fig. S5), even though the difference in perch diameter (1 versus 9 cm) imposed in the laboratory experiment was much greater than the differences in mean perch diameter observed between the source population and those of the experimental founder islands (4.6 cm and 2.6 to 4.5 cm for Iron Cay and the experimental founder islands, respectively).

Founder events generated among-island genetic and phenotypic differences that were similar in magnitude to those observed among populations on nearby islands (our reference islands), as well as among natural populations occurring throughout the Bahamas (tables S1 to S3, Fig. 3, and figs. S3 and S5). The strong genetic differentiation among founder islands, illustrated by allelic differentiation and F_{ST} values of similar magnitude to those seen among nearby reference islands, remained stable over the 4 years of the study (Mantel's $r = 0.86$ to 0.94 for correlations among pairwise F_{ST} matrices in 2006, 2007, and 2009, all $P < 0.01$; tables S1 and S2). The strong

imprint of the founder effect and the stability of among-population genetic differentiation occurred despite evidence for ongoing immigration. Assignment tests and pedigree analyses revealed that 11% of individuals sampled on experimental founder islands in 2007 and 2009 had multilocus genotypes consistent with being first-generation immigrants. In terms of phenotypic differentiation, the level of hindlimb divergence observed among experimental founder islands was only slightly less than that seen both among nearby reference islands and among natural populations from across the Bahamas (fig. S5). These results did not change when putative immigrants were removed from analyses. That the magnitude of the genetic and phenotypic differentiation produced by founder events rivals that seen among populations on natural islands indicates that founder effects may, as some have suggested, be an important cause of variation among islands (3, 4).

Our results indicate that founder effects persist, even in the face of substantial adaptive differentiation. The impacts of founder events on neutral genetic variation have long been recognized and are clearly evident here (25, 27) (tables S1 and S2 and Fig. 3), but the impact of founder effects on phenotypic divergence is still debated. In support of the selectionist school, we show that differentiation from the source population is mostly the result of adaptation to the new environmental milieu on the experimental islands (7–9, 16–18, 22). However, the imprint of the founder effect remains apparent, even as this adaptive divergence has occurred; indeed, variation among experimental founder islands at the present time is better explained by initial phenotypes than by current environmental conditions (Fig. 4, inset). What remains to be seen is whether—or for how long—these founder effects will persist in the face of natural selection. One might expect that just as selection has driven a decrease in hindlimb length from that of the source population in response to the narrower vegetation on the founder islands, it will ultimately also drive smaller-scale differentiation among islands as their lizard populations similarly adapt to the much smaller differences in perch diameter and erase the signal of the founder event. This prediction is not a foregone conclusion: Not only are habitat differences among experimental founder islands small, but the ultimate outcome is dependent on the interaction of changing demographic, environmental, and genetic factors.

We here show that founder effects can play an important role in the divergence of island populations, even as adaptation occurs. By measuring the phenotypic values of founders and their descendants, we have distinguished between the influences of the founder effect and of adaptation; thus, we provide clear evidence of a rarely characterized dimension of evolutionary divergence among populations.

References and Notes

1. P. R. Grant, Ed., *Evolution on Islands* (Oxford Univ. Press, Oxford, 1998).

2. R. G. Gillespie, D. A. Clague, Eds., *Encyclopedia of Islands* (Univ. of California Press, Berkeley, CA, 2009).
3. E. Mayr, in *Evolution as a Process*, J. Huxley, A. C. Hardy, E. B. Ford, Eds. (Allen & Unwin, London, 1954), pp. 157–180.
4. E. Mayr, *Animal Species and Evolution* (Harvard Univ. Press, Cambridge, MA, 1963).
5. H. L. Carson, *Stadler Genet. Symp.* **3**, 51 (1971).
6. A. R. Templeton, *Genetics* **94**, 1011 (1980).
7. J. A. Endler, *Natural Selection in the Wild* (Princeton Univ. Press, Princeton, NJ, 1986).
8. N. H. Barton, in *Evolution on Islands*, P. R. Grant, Ed. (Oxford Univ. Press, Oxford, 1998), pp. 102–123.
9. D. Schluter, *The Ecology of Adaptive Radiation* (Oxford Univ. Press, Oxford, 2000).
10. T. Price, *Speciation in Birds* (Roberts & Co., Greenwood Village, CO, 2007).
11. N. H. Barton, B. Charlesworth, *Annu. Rev. Ecol. Syst.* **15**, 133 (1984).
12. P. R. Grant, B. R. Grant, K. Petren, *Genetica* **112–113**, 359 (2001).
13. M. Travisano, J. A. Mongold, A. F. Bennett, R. E. Lenski, *Science* **267**, 87 (1995).
14. P. Simões et al., *Evolution* **62**, 1817 (2008).
15. J. A. Coyne, N. H. Barton, M. Turelli, *Evolution* **51**, 643 (1997).
16. D. N. Reznick, C. K. Ghalambor, *Genetica* **112–113**, 183 (2001).
17. S. M. Clegg et al., *Evolution* **56**, 2090 (2002).
18. M. T. Kinnison, N. G. Hairston Jr., *Funct. Ecol.* **21**, 444 (2007).
19. C. W. Kilpatrick, in *Mammalian Population Genetics*, M. H. Smith, J. Joule, Eds. (Univ. of Georgia Press, Athens, GA, 1981), pp. 28–59.
20. R. J. Berry, in *Evolution on Islands*, P. R. Grant, Ed. (Oxford Univ. Press, Oxford, 1998), pp. 35–50.
21. P. R. Grant, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 7818 (2002).
22. J. B. Losos, *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles* (Univ. of California Press, Berkeley, CA, 2009).
23. The patterns of lizard extinction were similar to those documented for Hurricane Floyd in (31).
24. D. A. Spiller, T. W. Schoener, *Ecology* **88**, 37 (2007).
25. M. Nei, T. Maruyama, R. Chakraborty, *Evolution* **29**, 1 (1975).
26. R. Chakraborty, M. Nei, *Evolution* **31**, 347 (1977).
27. C. L. Tarr, S. Conant, R. C. Fleischer, *Mol. Ecol.* **7**, 719 (1998).
28. J. B. Losos et al., *Evolution* **54**, 301 (2000).
29. T. W. Schoener, D. A. Spiller, J. B. Losos, *Ecol. Monogr.* **72**, 383 (2002).
30. D. Reznick, H. Rodd, L. Nunney, in *Evolutionary Conservation Biology*, R. Ferrière, U. Dieckmann, D. Couvet, Eds. (Cambridge Univ. Press, Cambridge, 2004), pp. 101–118.
31. T. W. Schoener, D. A. Spiller, J. B. Losos, *Science* **294**, 1525 (2001).

Acknowledgments: We thank B. Pinder, J. Pioviva-Scott, D. Steinberg, Y. Stuart, C. Thornber, and C. White for assistance; the National Geographic Society (grant 8704-09), NSF (grants DEB-0444763 and DEB-0949415), and Duke University for funding; and the Bahamas Ministry of Agriculture and the Bahamas Environment, Science & Technology Commission of the Ministry of the Environment for permission to conduct this research. This material was based on work supported by NSF while one of the authors (D.A.S.) was working at NSF. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of NSF.

Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1209566/DC1
Materials and Methods

Figs. S1 to S5
Tables S1 to S6
References (32–45)

9 June 2011; accepted 17 November 2011
10.1126/science.1209566