LETTERS

Immigration history controls diversification in experimental adaptive radiation

Tadashi Fukami[1], Hubertus J. E. Beaumont[2], Xue-Xian Zhang[2] & Paul B. Rainey[2]

Diversity in biological communities is a historical product of immigration, diversification and extinction[1–4], but the combined effect of these processes is poorly understood. Here we show that the order and timing of immigration controls the extent of diversification. When an ancestral bacterial genotype was introduced into a spatially structured habitat, it rapidly diversified into multiple niche-specialist types[5]. However, diversification was suppressed when a niche-specialist type was introduced before, or shortly after, introduction of the ancestral genotype. In contrast, little suppression occurred when the same niche specialist was introduced relatively late. The negative impact of early arriving immigrants was attributable to the historically sensitive outcome of interactions involving neutral competition[3] and indirect facilitation. Ultimately, the entire boom-and-bust dynamics of adaptive radiation were altered. These results demonstrate that immigration and diversification are tightly linked processes, with small differences in immigration history greatly affecting the evolutionary emergence of diversity.

MacArthur and Wilson's theory of island biogeography explains species diversity as a balance between immigration rate and extinction rate[6]. Although celebrated for simplicity and generality, this model leaves much variation in diversity unexplained[7]. Over recent years, two extensions to the basic theory have emerged: one recognizes that diversity is influenced not just by immigration rate, but also by immigration history (the order and timing of species arrivals)[8–11]; the other stresses the role of diversification (the evolutionary process of lineage splitting)[2–4,12]. Here we explore the interplay between immigration history and diversification.

Whereas historical contingency in evolution has been investigated[13–15], few studies have considered evolutionary effects of immigration history. For example, adaptive radiation has often been assumed to occur with the immigration of a single ancestral species[16]. In reality, immigration of multiple species in various orders and timings is possible[17,18]. Consider an ancestral species that colonizes an island from a distant mainland and subsequently diversifies into multiple species. It is likely that nearby islands will receive multiple species, both ancestral and derived, and that immigration history will vary among islands. Currently we know little about the implications that such variation in immigration has for diversity over evolutionary time. Some insight has arisen from phylogenetic analyses of community assembly[16,19,20], but these approaches provide little detailed information on the historical effects of immigration[18,20,21]. An ideal study would experimentally manipulate the timing of immigration events and observe directly the consequences for the evolutionary emergence of diversity.

Such experiments are difficult to perform on most organisms, but uniquely feasible with bacteria. In particular, experimental populations of *Pseudomonas fluorescens* SBW25 have been used to study adaptive radiation[5,22,23]. *P. fluorescens* populations founded from a single ancestral genotype diversify rapidly when introduced into a spatially structured aquatic microcosm[5]. Three main classes of genetically determined niche-specialist genotypes arise: the smooth morph class (SM), which resembles the ancestral type and primarily colonizes the liquid phase; the wrinkly-spreader morph class (WS), which forms biofilms at the air–liquid interface; and the fuzzy-spreader morph class (FS), which inhabits the microcosm bottom. These types are readily distinguishable by colony morphology[5], and considerable heritable variation exists within each class, particularly within WS (ref. 24). Moreover, it is easy to isolate and store these genotypes, and to regulate the rate at which they migrate to new microcosms. Because reproduction in *P. fluorescens* is entirely asexual, these genotypes are analogous to species in other organisms[22].

To investigate the effect of immigration history on the extent of diversification, the ancestral SM genotype (source SM population) and a derived WS genotype were introduced into spatially structured microcosms in different orders and at different intervals of time (see Methods). This design simulates natural situations in which a source population of a single ecological type colonizes an island of an archipelago and diversifies on that island and, subsequently, the source population and the new populations derived from it emigrate to nearby islands. In such situations, stochastic forces influencing the timing of dispersal make immigration history variable among islands. Our experiment included a control treatment in which the source SM was introduced by itself to new microcosms (see Methods). Because each of the immigrant populations is isogenic, any genotypic variation that arises after introduction can be attributed to evolutionary diversification by *de novo* mutation from the ancestral genotype. Furthermore, the use of a neutral genetic marker (see Methods) enables the origin of populations of each new genotype to be determined.

When introduced alone, the source SM population rapidly diversified into a FS population and multiple WS populations (Fig. 1). Four WS genotypes were distinguishable on the basis of heritable differences in colony morphology, which we refer to as small-WS, large-WS, wheel-WS and SM-like-WS. All new genotypes emerged by day four and coexisted with the source SM population for the duration of the experiment. This radiation is an expected outcome from previous studies[5].

When the source SM population and a derived WS population (small-WS) were introduced sequentially, different immigration histories resulted in striking differences in the extent of diversification (Fig. 2). When the source SM was introduced first, followed at least 24 h later by small-WS, both populations increased in density (Fig. 2g, h, m, n), but diversification from the ancestral SM (Fig. 2a, b) was not significantly different from that observed in the SM-only control (Fig. 1a). In contrast, little diversification was observed when

[1]Department of Zoology, University of Hawaii at Manoa, Honolulu, Hawaii 96822, USA. [2]School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand.

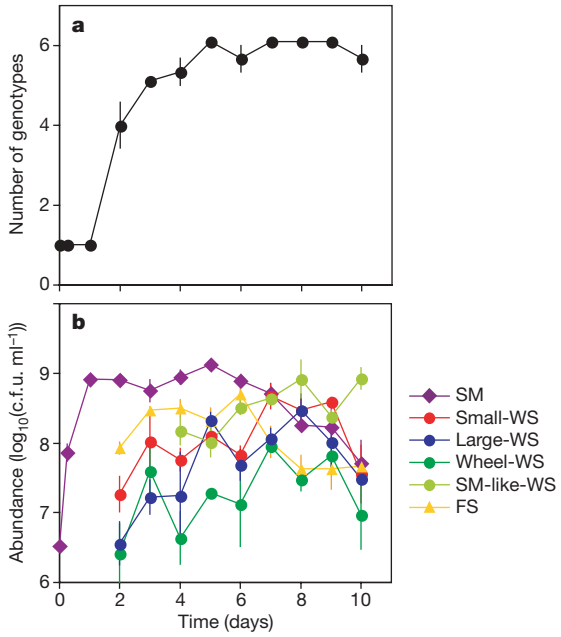

Figure 1 | Diversification of the ancestral SM genotype in spatially structured microcosms. **a**, Diversification; **b**, underlying population dynamics. Values shown are mean ± s.e.m. ($n = 3$); c.f.u., colony-forming units. See text for details of genotypes in **b**.

small-WS was introduced 6 h after (Fig. 2c), or before (Fig. 2d–f), introduction of the source SM population. Although both founding populations increased in density, diversification from the source SM was severely suppressed (Fig. 2i–l), while in most cases small-WS gave rise only to SM (Fig. 2o–r). Thus, slight differences in the early history of immigration had a large effect on the onset of adaptive radiation. This effect was solely caused by immigration history and was not due to any experimentally imposed difference in environmental conditions (compare refs 22, 25).

We further examined the dynamics of diversity over the longer term. To this end, each microcosm was homogenized after 10 days and a sample transferred to a fresh broth-filled vial. This process was repeated every 5 days (see Methods). Communities that were initially diverse showed a gradual decline in diversity and converged on a similar final diversity regardless of immigration history (Fig. 3). Boom-and-bust (or 'overshooting'[4,26]) dynamics in adaptive radiation, where species number first builds up and then declines, have been suggested to occur in a variety of taxa and habitats[4,16,25]. The radiation that occurred when the source SM population diversified in the absence of secondary invaders (Fig. 3) closely matched these dynamics (see Supplementary Discussion 1). What is even more intriguing, however, is the temporally contingent effect of secondary immigrants: early (but not late) arrival of small-WS entirely erased boom-and-bust dynamics (Fig. 3).

Changes in either the supply of mutants or the selective forces acting on these mutants (or a combination of both) are likely mechanisms of the observed effects. Three lines of evidence indicate that changes in mutation supply are not responsible. First, SM genotypes isolated from non-diversifying microcosms on day seven were indistinguishable from the wild type in terms of their capacity to generate, by mutation, WS and FS genotypes when introduced singly to fresh media (mean number of new genotypes emerged by day six ± s.e.m.: 4.83 ± 0.31 from the genotypes isolated from non-diversifying microcosms versus 4.67 ± 0.33 from the wild type; unpaired t-test, $t = 0.33$, $P > 0.74$). This result refutes the hypothesis that early arrival of small-WS induced genetic changes in SM that compromised their ability to diversify. Second, we repeated the immigration experiment using, in place of the ancestral SM genotype, a genetically derived variant (SM with a morph selection cassette, or SM^{msc}) that is phenotypically indistinguishable from SM, but engineered such that any mutation causing WS activates expression of a gene that encodes resistance to the antibiotic kanamycin (see Methods). The expression of kanamycin resistance (concomitant with mutation to WS) means that WS genotypes can be detected in a population dominated by other genotypes, simply by exposing the population to kanamycin. When small-WS was introduced early into populations founded by SM^{msc}, diversity was suppressed (as previously observed, for example, Fig. 2c, i, o) and no WS genotypes were detected when samples were spread on agar plates. However, exposure of these populations to kanamycin revealed that all microcosms contained multiple WS genotypes that were present at frequencies ranging from 1 in 10^4 to 1 in 10^6. Third, an analysis of the ability of

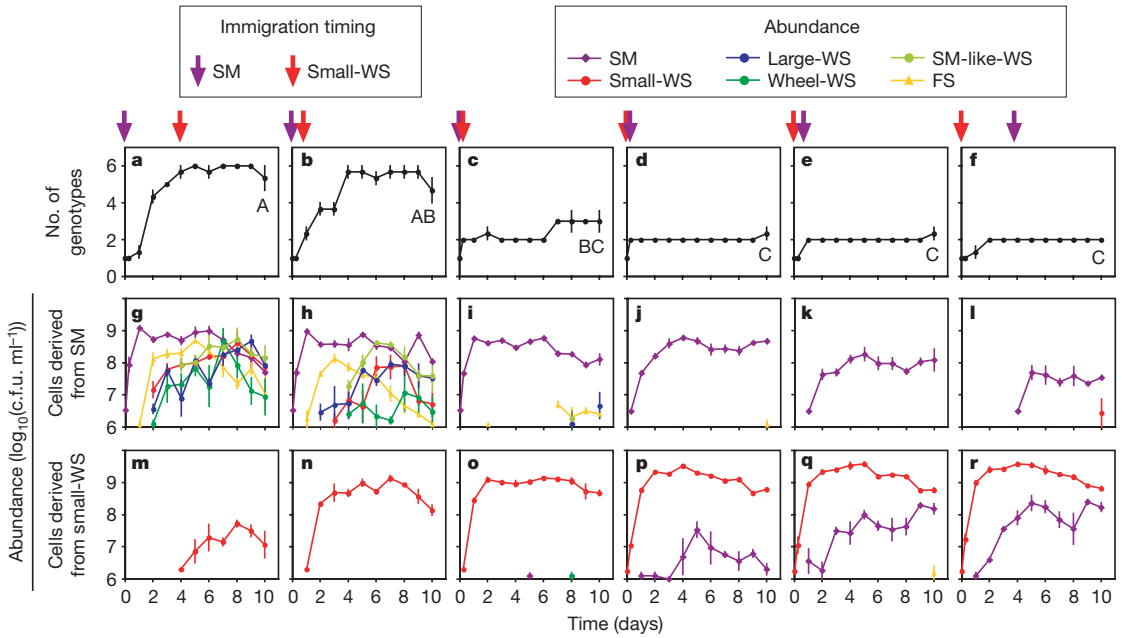

Figure 2 | Effects of immigration history. **a–f**, Effect on diversification; **g–r**, effect on population dynamics. Values shown are mean ± s.e.m. ($n = 3$). Treatments sharing the same letter (A, B, C) did not differ significantly ($P > 0.05$) in diversity on day 10 (Tukey's HSD test, $\alpha = 0.05$). Simpson and Shannon-Weaver diversity indices give similar patterns to those in **a–f**.

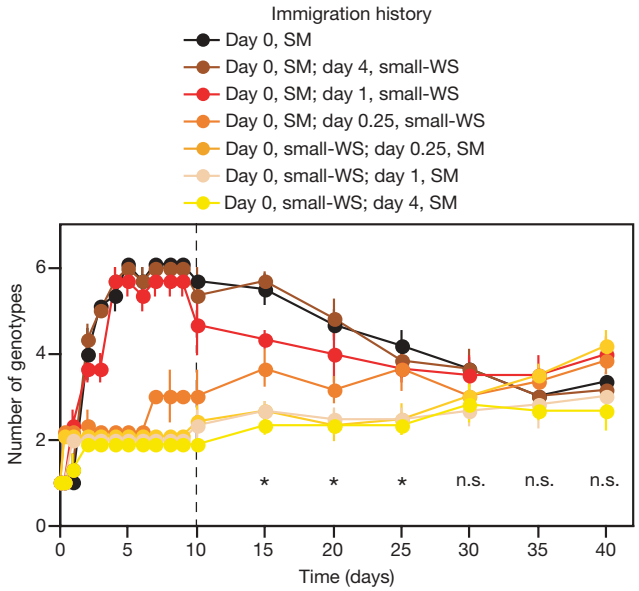

Figure 3 | Long-term changes in diversity. Dashed line indicates the start of the 5-day transfer treatments (see Methods). Immigration history had a significant effect ($P<0.05$) on diversity on days 15, 20 and 25 (asterisks), but not on days 30, 35, or 40 (n.s., not significant) (analysis of variance (ANOVA), $\alpha = 0.05$ with Bonferroni correction). Values shown are mean ± s.e.m. ($n = 3$ until day 10, and $n = 6$ after day 10).

these WS genotypes to invade populations of the ancestral SM genotype from the low frequency at which they were present in the non-diversifying microcosms showed a positive result within three days in nine out of ten instances. Together, these lines of evidence show that the suppression of diversification by early arriving immigrants is not a consequence of a marked reduction in mutation supply (see Supplementary Discussion 2).

To test the hypothesis that the introduction timing of small-WS affected the selective forces acting on the SM-derived WS genotypes, we inoculated fresh microcosms with mixtures of the four distinct WS genotypes at varying initial densities and determined the most abundant genotype after two days (see Methods). When all four genotypes were introduced at the same initial density, each became as abundant as the other three (Fig. 4). When small-WS received an advantage on account of an increased initial density, it dominated the microcosm and severely limited the growth of competing genotypes (Fig. 4). However, the other genotypes were equally capable of dominating when provided with an initial advantage (Fig. 4). These results indicate that WS genotypes are equally competitive (or neutral[3]): suppression of diversification by early arriving small-WS thus occurs as a consequence of pre-emptive colonization.

Suppression of FS by early arrival of small-WS is best explained by known competitive interactions among SM, WS and FS genotypes: FS can invade a population of SM or a mixed population of SM and WS, but not a population dominated by WS (refs 5, 23). Owing to its effect on oxygen and nutrient availability, WS has a competitive superiority over FS, such that it can exclude FS, but only in the absence of appreciable numbers of SM. In the presence of SM, WS is held in check on account of the negative frequency-dependent interaction between SM and WS, thus indirectly facilitating FS to increase in frequency[5,23]. This explains why the emergence of FS was suppressed only when small-WS was introduced early.

These mechanisms may be sufficiently general to also explain puzzling diversification patterns in other systems. For example, the filling of niches by species is thought to be deterministic in natural communities such that the same set of ecomorphs arise in multiple localities through immigration and diversification[4,14,16]. However, this expectation is not always met, even in well-described cases such as lizards on the Caribbean Islands[14], cichlids in African lakes[27], and land snails on the Hawaiian Islands[28]. In these cases, the extent of niche filling may depend stochastically on immigration history owing to indirect population interactions, as shown with FS in our study. Further, the extent of within-niche diversification is often variable among otherwise comparable communities of plants and arthropods on the Hawaiian[12], Canary[12,20] and other islands[16], which may be explained by the historically sensitive outcome of within-niche competition[3], as shown with WS here. Overall, immigration history may explain why boom-and-bust dynamics are common but not always observed, even within the same region[16].

Although it is clear that the ultimate sources of biodiversity are immigration and diversification[1–4,6], it has proven difficult to explain diversity as their additive product. Our results show that the extent of diversification can be altered greatly by even subtle differences in the early history of immigration. Such idiosyncrasies in the combined effect of immigration and diversification present a major obstacle in understanding diversity. Nevertheless, by determining the environmental conditions[10] and levels of community organization[29] under which history matters, progress can be made.

METHODS

Strains and culturing. *P. fluorescens* SBW25 (wild type) and *P. fluorescens* SBW25 *lacZ* (X.-X.Z. and P.B.R., unpublished results) were cultured in 25-ml universal vials with loose caps containing 6 ml of standard King's medium B (KB) in a 28 °C static incubator. After 4 days, cultures were plated onto KB agar and incubated for 2 days at 28 °C. Subsequently, a SM colony from the wild-type and a colony of small-WS from *P. fluorescens lacZ* were isolated, grown overnight

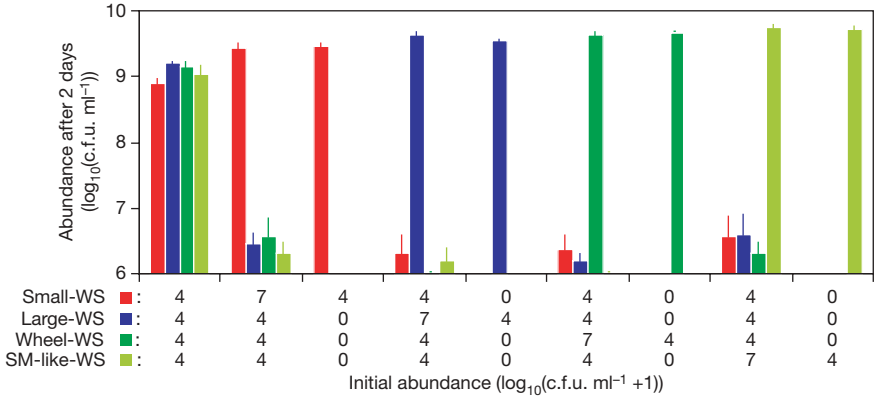

Figure 4 | Effect of the founding density of WS genotypes on the outcome of competition. Values shown are mean ± s.e.m. ($n = 3$). When the four genotypes were inoculated at the same founding density (10^4 cells ml^{-1}), they attained population sizes that were indistinguishable from one another (ANOVA, $F_{3,8} = 1.91$, $P > 0.21$). When any one of the genotypes received an advantage on account of an increased founding density (10^7 cells ml^{-1}), it became as abundant as when it was the sole colonizer (ANOVA, $F_{1,4} < 1.00$, $P > 0.37$) and severely limited the growth of the other genotypes.

in liquid KB medium at 28 °C in a 150 r.p.m. orbital shaker, and stored in 70% glycerol at −80 °C.

Manipulating immigration history. Microcosms were incubated statically with loose caps in 25-ml vials containing 6 ml KB at 28 °C. We inoculated the microcosms with SM and small-WS according to seven treatments of immigration history: (1) SM on day 0 (control), (2) SM on day 0 and small-WS on day 4, (3) SM on day 0 and small-WS on day 1, (4) SM on day 0 and small-WS on day 0.25 (that is, 6 h after SM), (5) small-WS on day 0 and SM on day 0.25, (6) small-WS on day 0 and SM on day 1, and (7) small-WS on day 0 and SM on day 4. We used 231 microcosms: 7 immigration history treatments × 3 replicates for each treatment × 11 destructive harvests. Each inoculation involved growing the stored SM and/or small WS for 16 h in liquid medium as described above and diluting so that $\sim 10^6$ cells could be introduced by transferring a volume of 20 µl into the microcosms at the pre-determined timings. Introduction of the immigrants was performed gently along the inside wall of the vials in order to minimize and standardize any resulting perturbation.

Determining genotype frequencies. Microcosms were destructively harvested 6 h after the start of the experiment and subsequently every 24 h. We determined cell densities of different genotypes by counting colonies after 2 days of growth on KB agar supplemented with 40 µg ml^{-1} 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal). We identified 6 heritable morphs: SM, small-WS, large-WS, wheel-WS, SM-like-WS and FS. Niche preference of these morphs was confirmed by observing growth of each genotype in static microcosms and establishing whether they mainly colonized the liquid phase (SM), the air–liquid interface (WS), or the bottom (FS) of the microcosms[5].

Examining long-term changes in diversity. On day 10, we transferred 6 µl of culture from each replicate of the main experiment to fresh medium. After five days, genotype frequencies were measured as described above, and 6 µl of the culture was transferred to fresh static microcosms. This procedure was repeated every 5 days until day 40.

Detecting low-frequency WS genotypes. SM^{msc} was constructed by integration of vector pUIC3 (ref. 30) harbouring the Tn*903* kanamycin resistance gene under the control of the *wss*-operon promoter (P_{wss}), into the chromosome of *P. fluorescens* SBW25 via homologous recombination. P_{wss} activity is upregulated through pleiotropic interactions in WS genotypes[24]. SM^{msc}-derived WS genotypes were detected by plating of approximately 10^6 cells from 10 independent non-diversifying microcosms onto selection medium (KB containing 30 µg ml^{-1} kanamycin, 0.0015% (w/v) Congo red and 60 µg ml^{-1} X-gal) and scored after two days. Congo red specifically stains WS colonies. Three distinct WS genotypes were identified and confirmed by re-streaking onto KB agar and by determination of the capacity of ten random WS genotypes to invade stationary-phase cultures of SM from the relative starting frequency at which they were found ($\sim 10^{-5}$). Nine out of these ten WS types were indeed capable of invading. As a control for the potential evolution of WS genotypes after plating on the selective medium, an equivalent number of cells from 6 independent 50-µl cultures (minimal selection for WS genotypes) were plated onto the selection medium. No Congo red binding colonies with WS morphologies were detected after six days of incubation, indicating that no evolution to WS occurred on the plates (see Supplementary Fig. 2).

Testing for the effect of initial densities on competitive outcome. Colonies of the four WS genotypes from day-7 plates of the SM-only inoculation treatment group were isolated and stored until use as described above. Each population was grown for 16 h in a shaken incubator, diluted as necessary, and introduced into static microcosms containing 6 ml KB at densities of 10^4 or 10^7 cells ml^{-1} (Fig. 4). Genotype frequencies were measured as described above after two days. Note that two days is long enough for populations to reach carrying capacity, but too short for evolutionary emergence of new types to confound the results.

Received 29 November 2006; accepted 29 January 2007.

1. Ricklefs, R. E. & Schluter, D. (eds) *Species Diversity in Ecological Communities: Historical and Geographical Perspectives* (Univ. Chicago Press, Chicago, 1993).
2. Losos, J. B. & Schluter, D. Analysis of an evolutionary species-area relationship. *Nature* **408,** 847–850 (2000).
3. Hubbell, S. P. *The Unified Neutral Theory of Biodiversity and Biogeography* (Princeton Univ. Press, Princeton, 2001).
4. Gillespie, R. G. Community assembly through adaptive radiation in Hawaiian spiders. *Science* **303,** 356–359 (2004).
5. Rainey, P. B. & Travisano, M. Adaptive radiation in a heterogeneous environment. *Nature* **394,** 69–72 (1998).
6. MacArthur, R. H. & Wilson, E. O. *The Theory of Island Biogeography* (Princeton Univ. Press, Princeton, 1967).
7. Connor, E. F. & McCoy, E. D. The statistics and biology of the species-area relationship. *Am. Nat.* **113,** 791–833 (1979).
8. Drake, J. A. Community-assembly mechanics and the structure of an experimental species ensemble. *Am. Nat.* **137,** 1–26 (1991).
9. Weiher, E. & Keddy, P. A. (eds) *Ecological Assembly Rules: Perspectives, Advances, Retreats* (Cambridge Univ. Press, Cambridge, 1999).
10. Chase, J. M. Community assembly: when should history matter? *Oecologia* **136,** 489–498 (2003).
11. Fukami, T. & Morin, P. J. Productivity-biodiversity relationships depend on the history of community assembly. *Nature* **424,** 423–426 (2003).
12. Emerson, B. C. & Kolm, N. Species diversity can drive speciation. *Nature* **434,** 1015–1017 (2005).
13. Travisano, M., Mongold, J. A., Bennett, A. F. & Lenski, R. E. Experimental tests of the roles of adaptation, chance, and history in evolution. *Science* **267,** 87–90 (1995).
14. Losos, J. B., Jackman, T. R., Larson, A., de Queiroz, K. & Rodriguez-Schettino, L. Contingency and determinism in replicated adaptive radiations of island lizards. *Science* **279,** 2115–2118 (1998).
15. Taylor, E. B. & McPhail, J. D. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus. Proc. R. Soc. B* **267,** 2375–2384 (2000).
16. Schluter, D. *The Ecology of Adaptive Radiation* (Oxford Univ. Press, Oxford, 2000).
17. Gillespie, R. G., Palumbi, S. R. & Croom, H. B. Multiple origins of a spider radiation in Hawaii. *Proc. Natl Acad. Sci. USA* **91,** 2290–2294 (1994).
18. Emerson, B. C. Evolution on oceanic islands: molecular phylogenetic approaches to understanding pattern and process. *Mol. Ecol.* **11,** 951–966 (2002).
19. Webb, C. O., Ackerly, D. D., McPeeck, M. A. & Donoghue, M. J. Phylogenies and community ecology. *Annu. Rev. Ecol. Syst.* **33,** 475–505 (2002).
20. Emerson, B. C. & Oromí, P. Diversification of the forest beetle genus *Tarphius* in the Canary Islands, and the evolutionary origins of island endemics. *Evolution* **59,** 586–598 (2005).
21. Losos, J. B. & Glor, R. E. Phylogenetic comparative methods and the geography of speciation. *Trends Ecol. Evol.* **18,** 220–227 (2003).
22. Kassen, R., Llewellyn, M. & Rainey, P. B. Ecological constraints on diversification in a model adaptive radiation. *Nature* **451,** 984–988 (2004).
23. Rainey, P. B. in *The Influence of Cooperative Bacteria on Animal Host Biology* (eds McFall-Ngai, M. J., Henderson, B. & Ruby, E. G.) 83–100 (Cambridge Univ. Press, Cambridge, 2005).
24. Goymer, P. *et al.* Adaptive divergence in experimental populations of *Pseudomonas fluorescens*. II. Role of the GGDEF regulator WspR in evolution and development of the wrinkly spreader phenotype. *Genetics* **173,** 515–526 (2006).
25. Doebeli, M. & Dieckmann, U. Speciation along environmental gradients. *Nature* **421,** 259–264 (2003).
26. Gavrilets, S. & Vose, A. Dynamic patterns of adaptive radiation. *Proc. Natl Acad. Sci. USA* **102,** 18040–18045 (2005).
27. Seehausen, O. African cichlid fish: a model system in adaptive radiation research. *Proc. R. Soc. Lond. B* **273,** 1987–1998 (2006).
28. Cowie, R. H. Variation in species diversity and shell shape in Hawaiian land snails: in situ speciation and ecological relationships. *Evolution* **49,** 1191–1202 (1995).
29. Fukami, T., Bezemer, T. M., Mortimer, S. R. & Van der Putten, W. H. Species divergence and trait convergence in experimental plant community assembly. *Ecol. Lett.* **8,** 1283–1290 (2005).
30. Rainey, P. B. Adaptation of *Pseudomonas fluorescens* to the plant rhizosphere. *Environ. Microbiol.* **1,** 243–257 (1999).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank P. Meintjes for assistance, and B. Emerson, R. Kassen and the members of the Rainey laboratory for comments. This work was supported by the Marsden Fund Council from government funding administered by the Royal Society of New Zealand, and by the Japan Society for the Promotion of Science.

Author Contributions T.F. developed the concepts, designed the main experiment with P.B.R., collected and analysed the primary data, and wrote the manuscript in conjunction with P.B.R. and H.J.E.B. H.J.E.B. and P.B.R. conceptualized the SM^{msc} genotype, which was constructed and validated by H.J.E.B. X.-X.Z. designed, constructed and validated *lacZ*-marked SBW25. H.J.E.B. and T.F. performed selection experiments.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to T.F. (tfukami@hawaii.edu).