Moreover, they are not expected to occur in birds according to one predominant theory of the evolution of genomic imprinting, and genes that are imprinted in mammals show ordinary bi-allelic expression in birds (*21*). We therefore conclude that species-assortative mating preferences in flycatcher hybrid zones are mainly due to Z-linked genes.

All three major components of reproductive isolation (species recognition, species-specific male traits, and hybrid incompatibilities) being Z linked in flycatchers should facilitate an evolutionary response to natural selection against hybridization. This is because genetic associations between the male and the female components of pre-zygotic barriers to gene flow, as well as between pre-zygotic and post-zygotic barriers, can easily be maintained (see supporting online text for further discussion of the flycatcher system). Our results suggest that some organisms may be prone to speciation through reinforcement because of the mediating role of the sex chromosomes. Compared to autosomally inherited species recognition, both sex linkage and sexual imprinting may allow incipient species to avoid a collapse in assortative mating during secondary contact and be less likely to succumb to gene flow and fusion (*9*). However, paternal sexual imprinting requires that females be socially exposed to their father, which is not always true even in birds. Conversely, because reduced hybrid fitness is commonly caused by sex-linked incompatibilities (*3*), sex linkage of species recognition might provide a general connection between key components of reproductive isolation, which facilitates adaptive speciation in the face of gene flow.

Sex-chromosome linkage of species-assortative female mate preferences may be widespread, but few previous studies have explicitly investigated the mechanism of species recognition in hybrid zones. Even fewer studies have provided additional information on the genetics of hybrid fitness and the preferred traits, or evidence for reinforcement (*22*–*25*). Nevertheless, disproportionately many genes involved in reproductive isolation seem to be located on the sex chromosomes (*15*, *26*, *27*). In Lepidoptera, which also have heterogametic females, sex-linked traits seem to be more associated with reproductive isolation than in other insects (*28*), and it has been suggested that ornaments and preferences for these ornaments evolve more readily in organisms with ZW than with XY sex chromosomes (*26*, *29*). Although speciation would benefit from any kind of linkage (or other recombination-suppressing mechanism) that can maintain these genetic associations, traits involved in pre-zygotic isolation may simply be more likely to occur on sex chromosomes than on autosomes and possibly more likely on Z than on X chromosomes (*27*). Sex chromosomes in general, and the Z in particular, may therefore be hotspots for speciation genes.

References and Notes

1. T. Dobzhansky, *Am. Nat.* **74**, 312 (1940).
2. M. R. Servedio, M. A. F. Noor, *Annu. Rev. Ecol. Syst.* **34**, 339 (2003).
3. J. A. Coyne, H. A. Orr, *Speciation* (Sinauer, Sunderland, MA, 2004).
4. M. Kirkpatrick, V. Ravigné, *Am. Nat.* **159**, 865 (2000).
5. J. Felsenstein, *Evol. Int. J. Org. Evol.* **35**, 124 (1981).
6. D. Ortíz-Barrientos, M. A. F. Noor, *Science* **310**, 1467 (2005).
7. C. ten Cate, D. R. Vos, *Adv. Stud. Behav.* **28**, 1 (1999).
8. D. E. Irwin, T. Price, *Heredity* **82**, 347 (1999).
9. M. R. Servedio, S. A. Sæther, G.-P. Sætre, *Evol. Ecol.*, in press, available at www.springerlink.com/content/ut50156832448324/.
10. A. J. Trickett, R. K. Butlin, *Heredity* **73**, 339 (1994).
11. M. R. Servedio, *Evol. Int. J. Org. Evol.* **54**, 21 (2000).
12. D. Ortíz-Barrientos *et al.*, *Genetica* **116**, 167 (2002).
13. M. R. Servedio, G.-P. Sætre, *Proc. R. Soc. London Ser. B* **270**, 1473 (2003).
14. D. W. Hall, M. Kirkpatrick, *Evol. Int. J. Org. Evol.* **60**, 908 (2006).
15. A. R. Lemmon, M. Kirkpatrick, *Genetics* **173**, 1145 (2006).
16. G.-P. Sætre *et al.*, *Nature* **387**, 589 (1997).
17. T. Veen *et al.*, *Nature* **411**, 45 (2001).
18. G.-P. Sætre *et al.*, *Proc. R. Soc. London Ser. B* **270**, 53 (2003).
19. See methods in supporting material on *Science* Online.
20. G.-P. Sætre, M. Král, S. Bureš, *J. Avian Biol.* **28**, 259 (1997).
21. M. J. O'Neill *et al.*, *Dev. Gen. Evol.* **210**, 18 (2000).
22. J. W. Grula, O. R. J. Taylor, *Evol. Int. J. Org. Evol.* **34**, 688 (1980).
23. P. R. Grant, R. B. Grant, *Biol. J. Linn. Soc.* **60**, 317 (1997).
24. V. K. Iyengar, H. K. Reeve, T. Eisner, *Nature* **419**, 830 (2002).
25. M. A. F. Noor *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 12084 (2001).
26. M. Kirkpatrick, D. W. Hall, *Evol. Int. J. Org. Evol.* **58**, 683 (2004).
27. V. B. Kaiser, H. Ellegren, *Evol. Int. J. Org. Evol.* **60**, 1945 (2006).
28. M. G. Ritchie, S. D. F. Phillips, in *Endless Forms: Species and Speciation*, D. J. Howard, S. H. Berlocher, Eds. (Oxford Univ. Press, Oxford, 1998), pp. 291–308.
29. H. K. Reeve, D. F. Pfenning, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1089 (2003).

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Microbial Population Structures in the Deep Marine Biosphere

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The analytical power of environmental DNA sequences for modeling microbial ecosystems depends on accurate assessments of population structure, including diversity (richness) and relative abundance (evenness). We investigated both aspects of population structure for microbial communities at two neighboring hydrothermal vents by examining the sequences of more than 900,000 microbial small-subunit ribosomal RNA amplicons. The two vent communities have different population structures that reflect local geochemical regimes. Descriptions of archaeal diversity were nearly exhaustive, but despite collecting an unparalleled number of sequences, statistical analyses indicated additional bacterial diversity at every taxonomic level. We predict that hundreds of thousands of sequences will be necessary to capture the vast diversity of microbial communities, and that different patterns of evenness for both high- and low-abundance taxa may be important in defining microbial ecosystem dynamics.

The interrogation of DNA from environmental samples has revealed new dimensions in microbial diversity and community-wide metabolic potential. The first analysis of a dozen polymerase chain reaction (PCR) amplicons of ribosomal RNA (rRNA) sequence from a mixed bacterioplankton population revealed the ubiquitous SAR11 cluster (*1*), and a recent environmental shotgun sequence survey of microbial communities in the surface ocean has identified 6.1 million predicted proteins (*2*, *3*). To realize the full potential of metagenomics for modeling energy and carbon flow, microbial biogeography, and the relationship between microbial diversity and ecosystem function, it is necessary to estimate both the richness and evenness of microbial population structures.

We used a tag sequencing strategy that combines the use of amplicons of the V6 hypervariable region of small-subunit (SSU) rRNA as proxies for the presence of individual phylotypes [operational taxonomic units (OTUs)] with massively parallel sequencing. Our goal was to provide assessments of microbial diversity, evenness, and community structure at a resolution two to three orders of magnitude greater than that afforded by cloning and capillary sequencing of longer SSU rRNA amplicons (*4*). We used this strategy to attempt an exhaustive characterization of the bacterial and archaeal diversity at two

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low-temperature diffuse flow vents, Marker 52 and Bag City, from Axial Seamount, an active volcano at 1520 m depth in the northeast Pacific Ocean (*5*, *6*). These vents host archaeal and bacterial communities originating from the subseafloor, local microbial mats, symbionts of vent macrofauna, and microorganisms from the surrounding seawater (*7*–*9*). Although new production from hydrothermal vents may correspond to as much as 25% of the total imported carbon flow in the deep sea (*10*), these globally distributed habitats remain relatively unexplored, and there are few descriptions of diversity, evenness, and dispersal of their endemic microbial populations.

Marker 52 and Bag City are less than 3 km apart, but differ markedly in chemical composition and appearance. Marker 52 was sampled on bare rock; Bag City vent fluids were sampled within a clump of tube worms. Both sites had microbial mats growing on rock and tube worm surfaces (fig. S1). Relative to Bag City and most other diffuse vents at Axial, Marker 52 has a higher $H_2S/\Delta T$ ratio, lower pH, and elevated alkalinity and iron levels (Table 1), all of which indicate a higher carbon dioxide content; Marker 52 fluids were effervescent at 1 atm (*9*).

We sequenced more than 900,000 archaeal and bacterial V6 amplicons from these two sites. Tags that differed by no more than 3% [generally considered to define microbial species (*11*)] were clustered (*12*) into OTUs to calculate rarefaction and nonparametric estimators (*13*). Taxonomic and statistical analyses revealed differences in community membership with very little overlap between the two sites (Table 2), which is particularly evident when comparing the fine structure of the communities (Fig. 1). For example, although ε-proteobacteria often dominate 10° to 80°C vent habitats, where they orchestrate the cycling of carbon, nitrogen, and sulfur (*14*), the richness and evenness of ε-proteobacterial families and genera are different at each site (Fig. 1). Nearly 6600 distinct ε-proteobacterial tag sequences accounted for 39% of bacterial amplicons, raising the estimate for total ε-proteobacterial diversity by at least one order of magnitude. Sequences identified as *Arcobacter* spp., a group of microaerophilic sulfur and hydrogen sulfide–oxidizing bacteria, dominated the ε-proteobacterial phylotypes at Bag City (FS312, Fig. 1), whereas sequences identified as *Sulfurovum* spp., a group of mesophilic microaerobes that use sulfur species as electron donors with nitrate or oxygen as electron acceptors, dominated Marker 52 (FS396, Fig. 1).

We hypothesize that the geochemical regimes shape the ε-proteobacterial community structure (Table 1) (*11*). A few highly abundant, specific tag sequences dominated each genus at each site, but extensive sampling revealed the presence of many less common and rare variants (Fig. 1). Microdiversity within groups of bacteria and archaea has been noted previously in the marine environment (*15*, *16*). It is clear that in some cases, these closely related organisms are ecologically distinct (*15*, *17*).

Nearly 6000 unique sequences from a data set of more than 215,000 V6 amplicon tags identified as archaeal defined more than 1900 phylotypes. The slope of the rarefaction curve (*18*) for the archaea became nearly asymptotic, and nonparametric statistical analyses estimated an ultimate richness of ~2700 archaeal phylotypes (Fig. 2 and Table 2). In contrast, despite examining nearly 690,000 tags identified as bacterial, rarefaction curves (Fig. 2) indicated that our sampling of bacterial richness was far from complete. We observed more than 30,000 unique bacterial sequences forming ~18,500 phylotypes, and nonparametric estimates predicted the presence of ~37,000 phylotypes (*13*) (Table 2), with steeply sloping rarefaction curves for many diverse classes, orders, and families (fig. S2). Even the dominant genera *Arcobacter* and *Sulfurovum* were incompletely sampled (fig. S2). The lower diversity of archaeal phylotypes agreed with other molecular surveys indicating that marine archaeal diversity is relatively limited (*19*); hence, our approach does not result in inflated richness estimates due to spurious data. Furthermore, extensive quality control of tag sequences ensured that the total error from PCR and pyrosequencing was less than 0.0025 per base and that sequencing error misassigned fewer than 1% of tags to phylotypes (*20*).

Table 1. Chemical and SSU rRNA tag characteristics of the two sites.

	FS312	FS396
Vent name	Bag City	Marker 52
Sample year	2003	2004
Volume filtered (ml)	1003	2000
Cells ml^{-1} (range)	1.21×10^5 (9.77×10^4 to 1.26×10^5)	1.57×10^5 (1.02×10^5 to 2.12×10^5)
Culturable* hyper/thermophilic heterotrophs per liter	140 to 4200	20 to 720
DNA recovered (μg)	0.9	2.4
Total number of archaeal V6 tag sequences†	200,199	16,428
Total number of bacterial V6 tag sequences†	442,058	247,662
Total number of ε-proteobacterial V6 tag sequences†	122,823	147,515
Depth (m)	1,537	1,529
Latitude and longitude	45.92°N, 129.99°W	45.94°N, 129.99°W
Average temperature (°C)	31.2	24.4
Maximum temperature (°C)	31.4	24.9
$H_2S/\Delta T$ (μmol kg^{-1} $°C^{-1}$)	7.2	18.9
pH	6.26	5.08
Mg (mmol/kg)	48.3	50.8
Alkalinity (meq/liter)	2.4	3.7
Mn (μmol/kg)	19.8	4.8
Fe (μmol/kg)	0.8	7.9
Silica (mmol/kg)	1.46	1.07

*Cultured at 70° or 90°C in 0.3% yeast extract and peptone with elemental sulfur; Ar headspace. †Trimmed reads that passed quality control [as described in (*11*)].

Table 2. Sequencing information and diversity estimates for all bacteria and archaea.

	Bacteria	Archaea
Total number of V6 tag sequences*	689,720	216,627
Total unique V6 tag sequences	30,108	5,979
Total OTUs at 3% difference (phylotypes)	18,537	1,931
Chao1 estimator of richness at 3% difference (95% CI)	36,869 (36,108 to 37,663)	2,754 (2,594 to 2,952)
ACE estimator of richness at 3% difference (95% CI)	37,038 (36,613 to 37,473)	2,678 (2,616 to 2,745)
Bray-Curtis similarity index at 3% difference†	0.08	0.01
Jaccard similarity index at 3% difference†	0.12	0.08

*Trimmed reads that passed quality control [as described in (*11*)]. †Similarity between communities at sites FS312 and FS396 on a scale of 0 to 1 (where 1 represents identical communities).

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Comparing each unique sequence to our V6 reference database revealed well-characterized taxa, as well as many unknown microbial phylotypes. The 10 most abundant sequences occurred more than 10,000 times and were exact matches to sequences in our database, indicating that our sampling was representative. Of the 36,725 unique sequences found at the two sites, 36,180 were represented by fewer than 100 tags; of these, 13,385 were >10% different and ~4000 were >20% different from known SSU rRNA genes. Many rare, divergent taxa account for most of the observed novel microbial diversity (*4*, *21*).

Although this study only examined samples at two sites in the deep ocean, it has important im-

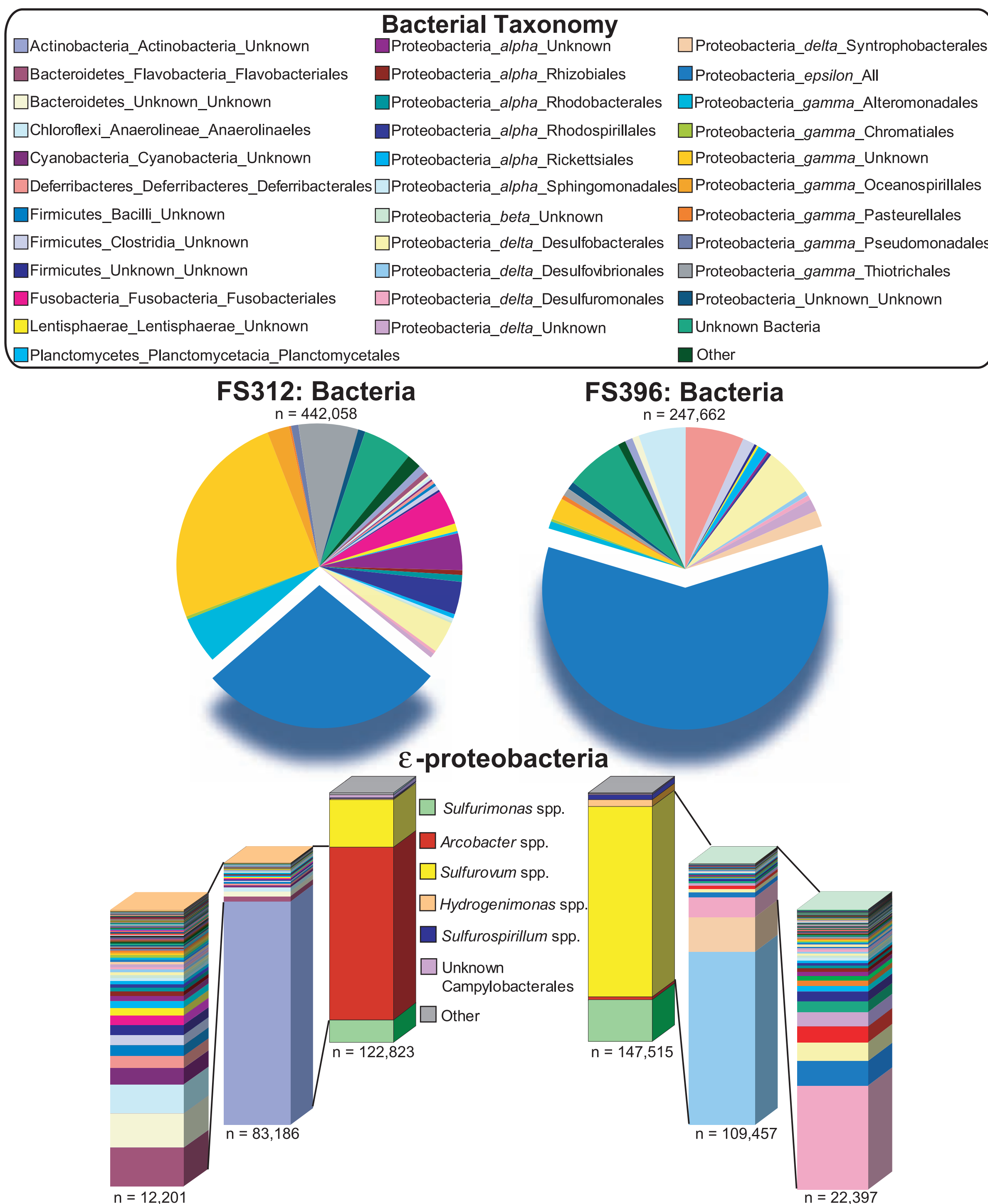

Fig. 1. Taxonomic breakdown of bacterial V6 tags from each vent. Pie charts show the Phylum_Class_Order distribution for taxonomically assigned tags that occurred more than 1000 times; the remaining tag sequences are grouped into "Other." The taxonomic distribution of ε-proteobacterial genera is shown in normalized histograms for each site, with further breakdown of the dominant ε-proteobacteria in additional histograms, with each color in the histograms representing a unique tag sequence. For FS312, the *Arcobacter* are expanded to the left with a histogram showing those tag sequences that occurred ≥10 times, followed by a histogram showing the diversity of tags that occurred 10 to 1800 times. For FS396, the *Sulfurovum* are expanded to the right, with a histogram showing those tag sequences that occurred ≥10 times, followed by a histogram showing the diversity of tags that occurred 10 to 8400 times. Nonparametric estimates suggested more than 900 phylotypes each of *Arcobacter* at FS312 and *Sulfurovum* at FS396.

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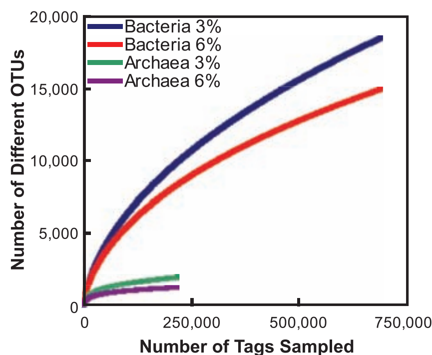

Fig. 2. Rarefaction curves for total bacterial and archaeal communities at the two sampling sites FS312 and FS396 at 3% and 6% difference levels.

plications for our ability to sample and identify all the ecologically relevant members of microbial communities in other high-diversity habitats, such as soils (*22*), microbial mats (*23*), and communities where low-abundance taxa may play crucial roles, such as the human microbiome. It provides a comparative population structure analysis with statistically significant descriptions of diversity and relative abundance of microbial populations. These large estimates of phylogenetic diversity at every taxonomic level present a challenge to large-scale microbial community genomic surveys. Metagenomic studies seek to inventory the full range of metabolic capabilities that define ecosystem function or to determine their context within assembled genomic scaffolds. Our results suggest that even the largest of published metagenomic investigations inadequately represent the full extent of microbial diversity, as they survey only the most highly abundant taxa (*11*).

In addition, the importance of microdiversity cannot be overlooked, and metagenomic community reconstructions from the two vents studied here would likely be largely chimeric assemblies of sequences from closely related phylotypes, which may mask important biological differences. Methods such as the massively parallel tag sequencing approach used here, combined with the multitude of other quantitative and descriptive tools now available to microbial ecologists, can serve as necessary accompaniments to metagenomic gene surveys as we strive to understand the impact of diversity on ecosystem function and long-term stability (*24*).

References and Notes

1. S. J. Giovannoni, T. B. Britschgi, C. L. Moyer, K. G. Field, *Nature* **345**, 60 (1990).
2. D. B. Rusch *et al.*, *PLoS Biol.* **5**, e77 (2007).
3. S. Yooseph *et al.*, *PLoS Biol.* **5**, e16 (2007).
4. M. L. Sogin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12115 (2006).
5. H. P. Johnson, R. W. Embley, *J. Geophys. Res.* **95**, 12689 (1990).
6. R. W. Embley, E. T. Baker, *Eos* **80**, 213 (1999).
7. J. A. Huber, D. A. Butterfield, J. A. Baross, *Appl. Environ. Microbiol.* **68**, 1585 (2002).
8. J. A. Huber, D. A. Butterfield, J. A. Baross, *FEMS Microbiol. Ecol.* **43**, 393 (2003).
9. D. A. Butterfield *et al.*, in *The Subseafloor Biosphere at Mid-Ocean Ridges,* W. S. D. Wilcock, E. F. DeLong, D. S. Kelley, J. A. Baross, S. C. Cary, Eds. (American Geophysical Union, Washington, DC, 2004), pp. 269–289.
10. A. Maruyama, T. Urabe, J. Ishibashi, R. A. Feely, E. T. Baker, *Cah. Biol. Mar.* **39**, 249 (1998).
11. See supporting material on *Science* Online.
12. P. D. Schloss, J. Handelsman, *Appl. Environ. Microbiol.* **71**, 1501 (2005).
13. A. Chao, *Scand. J. Stat.* **11**, 265 (1984).
14. B. J. Campbell, A. S. Engel, M. L. Porter, K. Takai, *Nat. Rev. Microbiol.* **4**, 458 (2006).
15. L. R. Moore, G. Rocap, S. W. Chisholm, *Nature* **393**, 464 (1998).
16. S. G. Acinas *et al.*, *Nature* **430**, 551 (2004).
17. G. Rocap, D. L. Distel, J. B. Waterbury, S. W. Chisholm, *Appl. Environ. Microbiol.* **68**, 1180 (2002).
18. D. M. Raup, *Paleobiology* **1**, 333 (1975).
19. R. Massana, E. F. DeLong, C. Pedros-Alio, *Appl. Environ. Microbiol.* **66**, 1777 (2000).
20. S. M. Huse, J. A. Huber, H. G. Morrison, M. L. Sogin, D. Mark Welch, *Genome Biol.* **8**, R143 (2007).
21. C. Pedros-Alio, *Trends Microbiol.* **14**, 257 (2006).
22. P. D. Schloss, J. Handelsman, *PLoS Comput. Biol.* **2**, e92 (2006).
23. R. E. Ley *et al.*, *Appl. Environ. Microbiol.* **72**, 3685 (2006).
24. E. F. DeLong, *Nat. Rev. Microbiol.* **5**, 326 (2007).
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Genetic Effects of Captive Breeding Cause a Rapid, Cumulative Fitness Decline in the Wild

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Captive breeding is used to supplement populations of many species that are declining in the wild. The suitability of and long-term species survival from such programs remain largely untested, however. We measured lifetime reproductive success of the first two generations of steelhead trout that were reared in captivity and bred in the wild after they were released. By reconstructing a three-generation pedigree with microsatellite markers, we show that genetic effects of domestication reduce subsequent reproductive capabilities by ~40% per captive-reared generation when fish are moved to natural environments. These results suggest that even a few generations of domestication may have negative effects on natural reproduction in the wild and that the repeated use of captive-reared parents to supplement wild populations should be carefully reconsidered.

Captive breeding was originally used as a form of conservation for the most critically endangered species, but is now widely used for the restoration of declining natural populations (*1*–*3*). In theory, captive-reared organisms may accumulate deleterious alleles that could hinder the recovery of natural populations (*3*–*6*). However, the extent to which captive-reared individuals contribute genetically to the restoration of natural populations is not known.

Hatchery programs for enhancing threatened populations of Pacific salmon and steelhead trout (*Oncorhynchus* spp.) release more than five billion juvenile hatchery fish into the North Pacific every year (*7*, *8*). Although most of these hatchery programs are meant to produce fish for harvest, an increasing number of captive breeding programs are releasing fish to restore declining natural populations (*8*, *9*). Hatchery fish breed in the wild, and many natural populations are affected by hatchery fish. The use of hatchery-reared fish as broodstock (parents of hatchery fish) for many generations has resulted in individuals that contribute less to the gene pool (are less fit), in comparison with wild fish, in natural environments (*10*–*12*). On the other hand, captive breeding programs that use local wild fish as broodstock are expected to produce hatchery fish having minimal differences in fitness from wild fish. Nevertheless, such captive-reared fish can be genetically distinct from wild fish for a variety of traits (*13*–*16*). Thus, it is a real concern that these fish will also have low fitness (reproductive success) in natural environments.

A two-generation pedigree of DNA-based parentage analyses of steelhead (*Oncorhynchus*

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