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 phyotypes, 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
11. See supporting material on Science Online.
25. We thank the NOAA Pacific Marine Environmental Laboratory Vents Program, the ROPOS Remotely Operated Vehicle, and S. Bolton for field support, and P. Schloss and L. Amaral Zettler for assistance in data analysis and primer design. Supported by NASA Astrobiology Institute Cooperative Agreement NNA04CC04A (MLS.), a National Research Council Research Associateship Award and L’Oréal USA Fellowship (J.A.H.), the Alfred P. Sloan Foundation’s IC3M field project, the W. M. Keck Foundation, and the Joint Institute for the Study of the Atmosphere and Ocean under NOAA Cooperative Agreement NA17RJ1232, Contribution 1388. This is NOAA Pacific Marine Environmental Laboratory Contribution 3047. The new sequences reported in this paper have been deposited in the NCBi Short Read Archive under accession numbers SRA000195 and SRA000196. The zip file available for download via http from jesp.mbl.edu/research_supplements/ g454/20070822-private-supplemental.zip contains all the fasta-formatted trimmed reads used in the analyses.

Supporting Online Material
www.sciencemag.org/cgi/content/full/318/5847/97/DC1
Materials and Methods
SOM Text
Figs. S1 and S2
References
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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 tshawytscha) and salmon (Oncorhynchus spp.) were constructed from the offspring of captive-reared parents with wild-caught parents (17). The pedigrees included four generations of captive-reared families in which the parents were reared in captivity and then released into the wild in the summer of 1999. The genetic contributions of the captive-reared fish were determined using microsatellite markers (18). The microsatellite loci used in this study were selected based on their polymorphism and informativeness (19). The captive-reared fish were released into the wild at the age of one year, and the wild-caught fish were caught from the wild at the age of two years. The offspring of these captive-reared and wild-caught fish were then captured from the wild at the age of three years.

The results of these genetic analyses were used to determine the contribution of the captive-reared fish to the offspring of these captive-reared and wild-caught fish were then captured from the wild at the age of three years.

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mylekiss) in the Hood River in Oregon (U.S.A.) showed that the first generation of captive-reared fish had natural reproductive success indistinguishable from that of wild fish in two out of three run-years (17). (Each run-year begins when parents arrive at the river to spawn.) This comparison, however, neglected the fact that captive-reared and wild individuals experience different environments as juveniles, which might affect mating behaviors, fecundity, and/or fertility (18). Therefore, it is difficult to disentangle environmental effects from genetic effects of a difference or lack of difference in reproductive success (17).

In this study, we investigated the strength of genetic effects of domestication on the reproductive success of captive-reared individuals in the wild. Confounding environmental effects were avoided by comparing captive-reared individuals with different histories of captive breeding in the previous generation (Fig. 1). We reconstructed a three-generation pedigree of the winter-run steelhead in the Hood River (19) and compared adult-to-adult reproductive success (number of wild-born, adult offspring per parent) of two types of captive-reared fish (designated C): captive-reared fish from two wild-born parents (C[WXW]), and captive-reared fish from a wild-born parent and a first-generation captive-reared parent (C[CxW]). C[CxW] and C[WXW] were born in the same year, reared in the same hatchery without distinction, and released at the same time. Both fish originated from the same local population, so we can also exclude the influence of local origin. The only difference between them is half of the genome. The half genome in C[CxW] was inherited from the captive-reared parent and experienced captivity for two consecutive generations (during the egg-to-juvenile development). The other half in C[CxW] was from the wild parent and experienced captivity for one generation (C[CxW] itself). In contrast, the entire genome of the C[WXW] experienced captivity for one generation. Thus, by comparing C[CxW] with C[WXW], we were able to evaluate the effect of a single extra generation of captive rearing on subsequent reproductive success in the wild, while controlling for the effect of rearing environment (Fig. 1).

We estimated the reproductive success of 547 C[CxW] and 193 C[WXW] over three run-years (1998–2000) (19). On the basis of the parentage analysis, we assigned 355 wild-born, returning adult offspring to at least one of their C[CxW] or C[WXW] parents (Table 1). Our estimate of relative reproductive success (RRS) with an unbiased method (20) revealed that the overall reproductive success of C[CxW] is 55% that of C[WXW] ($P = 0.009$ by one-tailed permutation tests). We also compared the reproductive success of C[CxW] and C[WXW] from single cohorts (i.e., using only 3-year-olds at the time of spawning) (Table 1). In this comparison, environmental differences were eliminated because both types of hatchery fish were born, returned, and spawned in the same environments in the same year. The smaller sample size resulted in lower power, but the overall estimate was very similar to the above result (single-cohort RRS of C[CxW] to C[WXW] = 0.609, $P = 0.042$).

In addition to comparing reproductive success between C[WXW] and C[CxW], we also compared the reproductive success of these captive-reared fish to that of wild-born fish (W) returning in the same run-years (1998–2000). Overall RRS of C[WXW] to W was 0.595 and that of C[CxW] to W was 0.310 (both $P < 0.001$, (Table S1)). Our estimates of RRS for C[WXW] can be compared with those from our previous study of run years 1995–1997 (17) (Table S1). Interestingly, the estimate from run years 1998–2000 was significantly lower than the average RRS ~ 1 estimated from run-years 1995–1997 (17) (Fig. 2A). One possible explanation for this difference is presence of C[CxW] on the spawning grounds in 1998–2000. For example, reproductive interaction between C[CxW] and C[WXW] might reduce the average reproductive success of C[WXW] if C[WXW] tend to mate more with C[CxW] than with W. Another possibility is nonadditive fitness effects such that mating between hatchery fish results in lower fitness than expected. In our data, nonrandom mating was supported by a test of independence ($P < 0.001$ for all three run-years (table S2)). However, an excess of observed mating was found between wild parents, not between captive-reared parents. This might indicate both nonrandom mating (WXW and CxC mating preferences) and nonadditive fitness effects (i.e.,

**Fig. 1.** Distribution of run-years in which captive-reared fish and their wild-born offspring returned. Numbers in a circle represent a run-year of parents (top) and a brood-year of their offspring (bottom). The percentage on each arrow represents the proportion of adults that return in each subsequent year, which differs between captive-reared fish (dotted line) and wild fish (solid line). C[CxW] were iteratively created from wild individuals and the first generation of captive-reared individuals that returned in run-year 1995; subsequent C[CxW] individuals were created from those individuals returning in 1996 and so forth. These first-year C[CxW] fish returned to spawn mostly in run-year 1998, and we estimated their reproductive success by matching them to the wild-born offspring that returned in run-year 2001–2004.

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**Table 1**

<table>
<thead>
<tr>
<th>Run-Year</th>
<th>Gender</th>
<th>Parentage</th>
<th>Proportion Returning</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>Male</td>
<td>C[xW]</td>
<td>0.41</td>
</tr>
<tr>
<td>1999</td>
<td>Female</td>
<td>C[CxW]</td>
<td>0.82</td>
</tr>
<tr>
<td>2000</td>
<td>Male</td>
<td>C[WXW]</td>
<td>0.31</td>
</tr>
</tbody>
</table>

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*Note:* C[WXW] and C[CxW] refer to captive-reared fish from wild parents and captive-reared parents, respectively. The table includes only those parents that returned in each run-year.
low fitness of CxC), although analyses of reproduct- 
itive success between crosses did not show the 
presence of nonadditive genetic effects [RRS of 
[C(CxW) × C(WxW)] to [W × C(WxW)] = 1.1 in 
run-year 2000, \( P = 0.878 \) (table S3)]. Over six 
run-years of data (1995–2000), four of six years 
showed lower fitness of C[WxW] (overall RRS of 
C[WxW] to \( W = 0.848, P < 0.001 \)).

One factor we cannot completely exclude in 
these comparisons is nongenetic grandparental 
effects, which have been demonstrated in various 
organisms, including fish (21–24). However, 
known grandparental effects are mostly female-
specific (i.e., grandmaternal egg effects). The re-
productive success of C(xC) did not depend 
on the sex of the captive-reared parent (overall 
RRS of C[CxW] with a captive-reared mother to 
C[CxW] with a captive-reared father = 1.009, \( P = 0.81 \)). Similarly, there were no notice-
table maternal effects on the reproductive 
success when hatchery and wild fish mated in 
the wild, either in this study or in our previous 
study [i.e., number of resulting offspring did 
not depend on which type of fish was the 
mother (table S1) (17)]. Thus, the grandpa-
rental effect is less likely in this case, and the 
most likely explanation for the fitness decline is a 
genetic disadvantage of C[CxW] resulting 
from the half genome exposed to artificial en-
vironments for an additional generation.

Our data suggest a sharp decline in repro-
ductive success follows a very short time in 
captivity (Fig. 2A). We also conducted a meta-
analysis to compare our data with those available 
for four hatchery stocks for which we know the 
number of generations in hatcheries (19, 25). 
These data fit very well on an exponentially 
decreasing curve (Fig. 2B), despite the fact that the 
previous data include RRS estimates using 
different species and methods and that they are 
subject to confounding environmental effects 
(19, 25). It shows 37.5% fitness decline per 
captive-reared generation, suggesting that the 
fitness decline of captive-reared fish can be remark-
ably fast. Because any purely environmental 
effects should not accumulate over time, the 
continued decline with generations in captivity 
(Fig. 2) further supports genetic effects as the 
cause.

The evolutionary mechanism causing the 
fitness decline remains unknown. We suspect 
that unintentional domestication selection and 
relaxation of natural selection, due to artificially 
modified and well-protected rearing environ-
m ents for hatchery fish, are probably occurring 
(SOM text). Considering the mating scheme for 
C[CxW] and the generation time for the fit-
ness decline, however, inbreeding depression 
and accumulation of new mutations should not 
 affect these results. Regardless, our data 
demonstrate how strong the effects can be and how 
quickly they accumulate. To supplement decline-
ing wild populations, therefore, repeat use of 
captive-reared organisms for reproduction of 
captive-reared progenies should be carefully 
reconsidered.

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Glia Promote Local Synaptogenesis Through UNC-6 (Netrin) Signaling in C. elegans

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Neural circuits are assembled through the coordinated innervation of pre- and postsynaptic partners. We show that connectivity between two interneurons, AIY and RIA, in Caenorhabditis elegans is orchestrated by a pair of glial cells that express UNC-6 (netrin). In the postsynaptic neuron RIA, the netrin receptor UNC-40 (DCC, deleted in colorectal cancer) plays a conventional guidance role, directing outgrowth of the RIA process ventrally toward the glia. In the presynaptic neuron AIY, UNC-40 (DCC) plays an unexpected and previously uncharacterized role: It cell-autonomously promotes assembly of presynaptic terminals in the immediate vicinity of the glial cell endfeet. These results indicate that netrin can be used both for guidance and local synaptogenesis and suggest that glial cells can function as guideposts during the assembly of neural circuits in vivo.

Neural circuit formation requires an intricate orchestration of multiple developmental events, including cell migration, axon guidance, dendritic growth, synaptic target selection, and synaptogenesis (1–3). These developmental events are coordinated in pre- and postsynaptic neuronal partners to form the functional neural circuits that underlie behaviors. Although the organization and specificity of these neural circuits is well documented, the cellular and molecular mechanisms that underlie their precise development are not well understood.

To explore how precise neural connectivity is achieved, we studied the synaptic connections between two interneurons in the C. elegans brain: presynaptic AIY and postsynaptic RIA. These two interneurons navigate complex cellular environments, discriminating among multiple potential targets before finding and innervating each other at a discrete region of their respective processes (4). We generated single-cell fluorescent markers to visualize AIY-RIA connectivity in vivo and observed a discrete clustering of presynaptic AIY markers in a segment of the process we termed zone 2. This zone appears to be the specialized presynaptic region where AIY forms synapses onto RIA, as well as RIB and AIZ neurons. First, the fluorescently labeled presynaptic proteins RAB-3, ELKS-1, and SYD-2 are all more concentrated in zone 2 than in other regions of the axon (Figs. 1A and 2B and fig. S4A). Second, these markers cluster at the exact location at which AIY to RIA synapses are seen in electron micrographs of wild-type animals (fig. S1M) (5). Third, this region has a wider diameter than other regions of the axon, a property that we found to be uniquely associated with the presynaptic region of AIY in electron micrographs (fig. S1, A and M to Q). These combined properties were taken as evidence of presynaptic differentiation and were very reproducible across animals (Fig. 1 and fig. S1).

Reconstructions of electron microscopy (EM) micrographs (5) revealed that AIY has three distinct anatomical regions throughout its process: a segment proximal to the AIY cell body that is devoid of synapses (zone 1); the synapse-rich region where AIY forms synapses onto RIA, AIZ, and RIB just as the AIY process turns dorsally (zone 2); and a distal axon segment within the nerve ring that has four to eight small presynaptic specializations (zone 3).

To identify the molecular signals that direct this precise innervation, we performed a visual genetic screen for mutants with an abnormal synapse distribution in AIY. From this screen, we isolated the wy81 mutation, an allele of unc-40 (fig. S2). UNC-40 (DCC, deleted in colorectal cancer) is a transmembrane immunoglobulin superfamily protein that is a receptor for the axon guidance molecule UNC-6 (netrin) (6, 7). unc-40 animals had no detectable axon guidance defects in AIY except for an axon truncation defect observed in 7.8% of the animals (n = 153 animals; fig. S3). However, they showed a highly penetrant defect in the presynaptic specialization of AIY at zone 2: 95.3% of unc-40(wy81) animals displayed a severe reduction of active zone markers ELKS-1:YFP (yellow fluorescent protein) and SYD-2:GFP (green fluorescent protein) and a synaptic vesicle marker, mCherry::RAB-3, in zone 2 (n = 128 animals; Fig. 2, A to K, and fig. S4). In addition, the AIY axon diameter in zone 2 failed to widen into the characteristic presynaptic varicosity seen in wild-type animals (fig. S1). By contrast, in the more-dorsal zone 3 synaptic regions, unc-40 animals had normal or increased levels of synaptic vesicle proteins and a normal or increased diameter (Fig. 2, F to I, and fig. S1). These defects suggest a specific defect in the presynaptic differentiation of AIY in zone 2, although a detailed analysis of AIY synaptic ultrastructure and function could

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