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Science 318, 100 (2007);
DOI: 10.1126/science.1145621

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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
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 Associate Award and L’Oréal USA Fellowship (J.A.H.), the Alfred P. Sloan Foundation’s CoMM 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 NCBJ Short Read Archive under accession numbers SRA0004195 and SRA000196. The zip file available for download via http://jspc.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
19 June 2007; accepted 28 August 2007
10.1126/science.1146689

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 thremelus)
mykiss) 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 only 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, reared, 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[WxW] and C[CxW] might reduce the average reproductive success of C[WxW] if C[CxW] 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 CxW mating preferences) and nonadditive fitness effects (i.e.,

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**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.
low fitness of CxC), although analyses of reproductive success between crosses did not show the presence of nonadditive genetic effects [RRS of \([C(CxW) \times C(WxW)]\) to \([W \times C(WxW)]\) = 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 reproductive success of C[CxW] 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 noticeable maternal effects on the reproductive success when hatchery and wild fish mated in the wild, either in this study or in our previous study, number of resulting offspring did not depend on which type of fish was the mother (table S1) (17). Thus, the grandparental 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 environments for an additional generation.

Our data suggest a sharp decline in reproductive 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 declining 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 remarkably 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 environments for hatchery fish, are probably occurring (SOM text). Considering the mating scheme for C[CxW] and the generation time for the fitness 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 declining wild populations, therefore, repeat use of captive-reared organisms for reproduction of captive-reared progenies should be carefully reconsidered.

References and Notes
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, AY 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 AY, 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 endfoot. 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 AY 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 AY-RIA connectivity in vivo and observed a discrete clustering of presynaptic AY markers in a segment of the process we termed zone 2. This zone appears to be the specialized presynaptic region where AY 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 isolated from the AY process: a segment proximal to the AY cell body and three distinct anatomical regions throughout its process: a segment proximal to the AY cell body that is devoid of synapses (zone 1); the synapse-rich region where AY forms synapses onto RIA, AIZ, and RIB just as the AY 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 AY. From this screen, we isolated the wy781 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 AY 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 AY at zone 2: 95.3% of unc-40 (wy81) animals displayed a severe reduction of active synapse distribution in AY. 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 AY 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 AY 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 AY 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 AY in zone 2, although a detailed analysis of AY synaptic ultrastructure and function could have been performed.