EVOLUTION

Contrasting genomic shifts underlie parallel phenotypic evolution in response to fishing

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Humans cause widespread evolutionary change in nature, but we still know little about the genomic basis of rapid adaptation in the Anthropocene. We tracked genomic changes across all protein-coding genes in experimental fish populations that evolved pronounced shifts in growth rates due to size-selective harvest over only four generations. Comparisons of replicate lines show parallel allele frequency shifts that recapitulate responses to size-selection gradients in the wild across hundreds of unlinked variants concentrated in growth-related genes. However, a supercluster of genes also rose rapidly in frequency and dominated the evolutionary dynamic in one replicate line but not in others. Parallel phenotypic changes thus masked highly divergent genomic responses to selection, illustrating how contingent rapid adaptation can be in the face of strong human-induced selection.

Human actions cause rapid evolutionary change in many species (*1*, *2*), but the underlying genomic basis remains poorly understood. Prime examples of human-driven evolution come from fisheries, as the selection pressure imposed by intense harvest has caused pronounced shifts in growth rates and reproductive timing in many commercially important stocks, potentially reducing yields and impeding recovery from overfishing (*3*, *4*). Fishing has been shown to change gene expression, genetic diversity, and allele frequencies at candidate markers (*5*–*8*), but the overall magnitude and extent of genomic change and the repeatability of response patterns remain unclear, hampering our ability to predict the consequences for fish stock resilience to continually changing fishing regulations and new challenges imposed by climate and other environmental change.

Theory and empirical studies suggest that rapid adaptation can occur through two broadly different paths. In classic sweep scenarios, phenotypic shifts are caused by large allele frequency changes at one locus or a few loci (*9*, *10*), which may quickly erode the genetic variation needed for reversal or other adaptive responses. In contrast, complex quantitative traits have traditionally been assumed to evolve through small allele frequency shifts across many loci (*11*), which should better retain functionally important variation and, to a lesser extent, compromise the ability of populations to adapt to future conditions. Both types of response patterns have been observed across experimental evolution studies in small mammals, insects, plants, and microbes (*12*, *13*). Yet, despite the important implications for the future evolutionary potential of species, we know little about how predictable genomic responses to pervasive human-induced selection are in large populations that harbor high levels of standing genetic variation, which can serve as raw material to fuel rapid adaptation.

We have returned to an influential experiment that demonstrated rapid evolution in response to size-selective fishing (*14*). The experiment subjected six populations each of ~1000 adult Atlantic silversides (*Menidia menidia*, a small estuarine fish) to 90% harvest every generation. In two replicate populations, the individuals left to reproduce were the smallest 10% (in body length, hereafter the "down-selected" lines). In two other populations, the largest 10% were left to reproduce each generation (hereafter the "up-selected" lines), and another two populations were controls (the 90% mortality was random with respect to size). After only five generations, fish from the up-selected lines were, on average, 25% longer than fish from the down-selected lines (Fig. 1), resulting in an almost twofold difference in average weight (*14*).

We used low-coverage (~1.3× coverage per individual) whole-genome sequencing of frozen fish from the experiment to examine the genomic basis underlying these phenotypic shifts. We sequenced 75 individuals from the source pool used to establish the experiment (offspring from hundreds of wild-caught parents, generation 0) and 48 to 50 individuals from each of the six populations in generation 5 (fig. S1 and table S1). The species is distributed along the east coast of North America, where a shorter growing season has driven the evolution of faster growth in northern regions (*15*, *16*). For comparison, we therefore also sequenced 42 to 50 individuals from each of four wild populations across this natural cline (table S2). In the absence of a reference genome, we mapped the genomic reads to a comprehensive reference transcriptome (*17*) to examine exome-wide patterns of change across 2.36 million single-nucleotide polymorphisms (SNPs) (*18*).

As expected, we observed a clear reduction in genetic diversity in all captive populations.

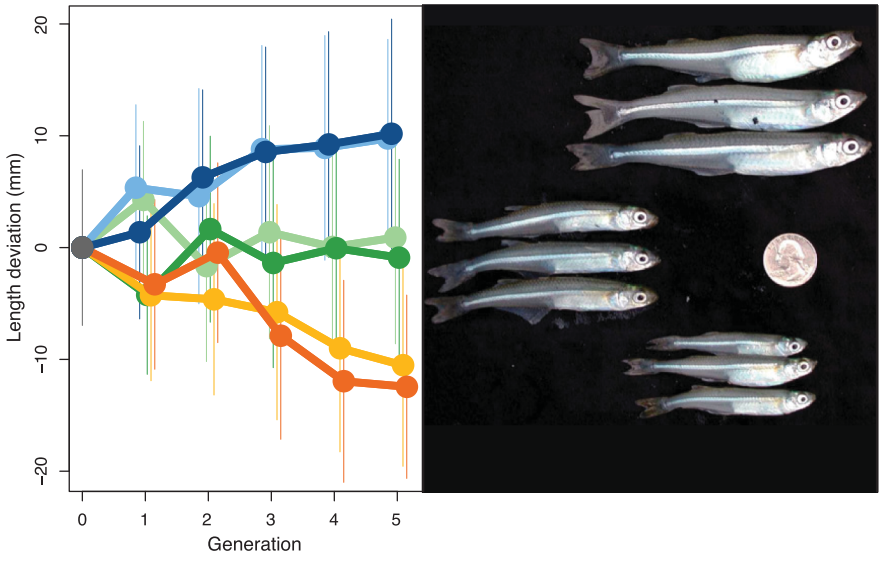

Fig. 1. Observed shifts in adult size. Trends across generations in mean length at harvest (standardized as the difference from the mean of the control populations in each generation) ± the standard deviations in up-selected (blue shades), down-selected (yellow and orange shades), and control populations (green shades).

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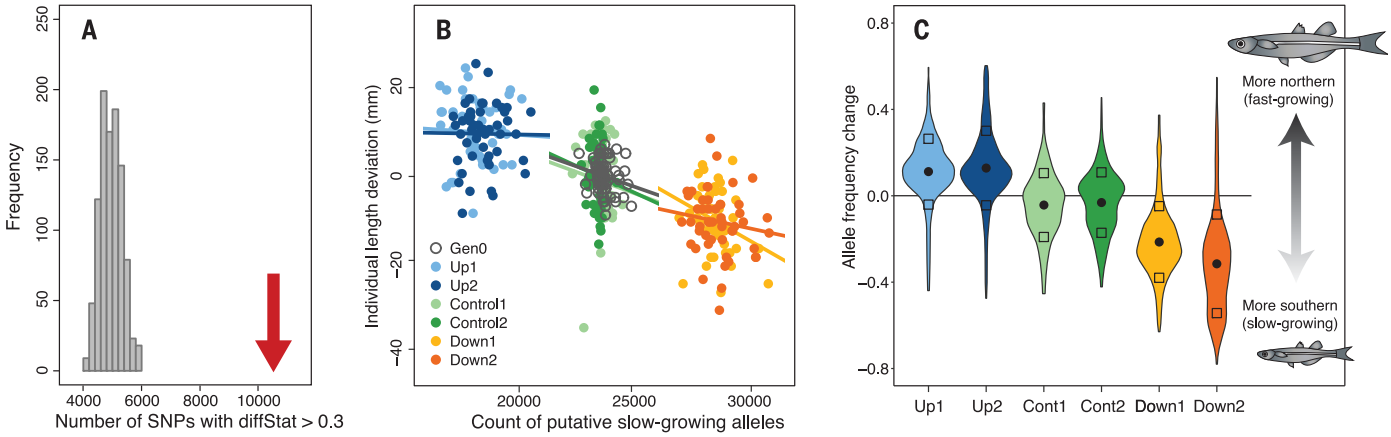

Fig. 2. Parallel genomic shifts among replicate treatments. (**A**) The number of SNPs with diffStat > 0.3 (red arrow) was much higher than observed in any of the 1000 simulated datasets (gray histogram). (**B**) Individual standardized length at harvest was negatively correlated with the number of putative slow-growing alleles found in each fish across the SNPs in the top 1% of the diffStat distribution. Regression lines show the slope in each population. (**C**) The distribution of change in frequency of the northern (putatively fast-growing) allele in each population across the 357 top-diffStat SNPs that also show strong genetic differentiation (F_{ST} > 0.5) in the wild. Cont, control.

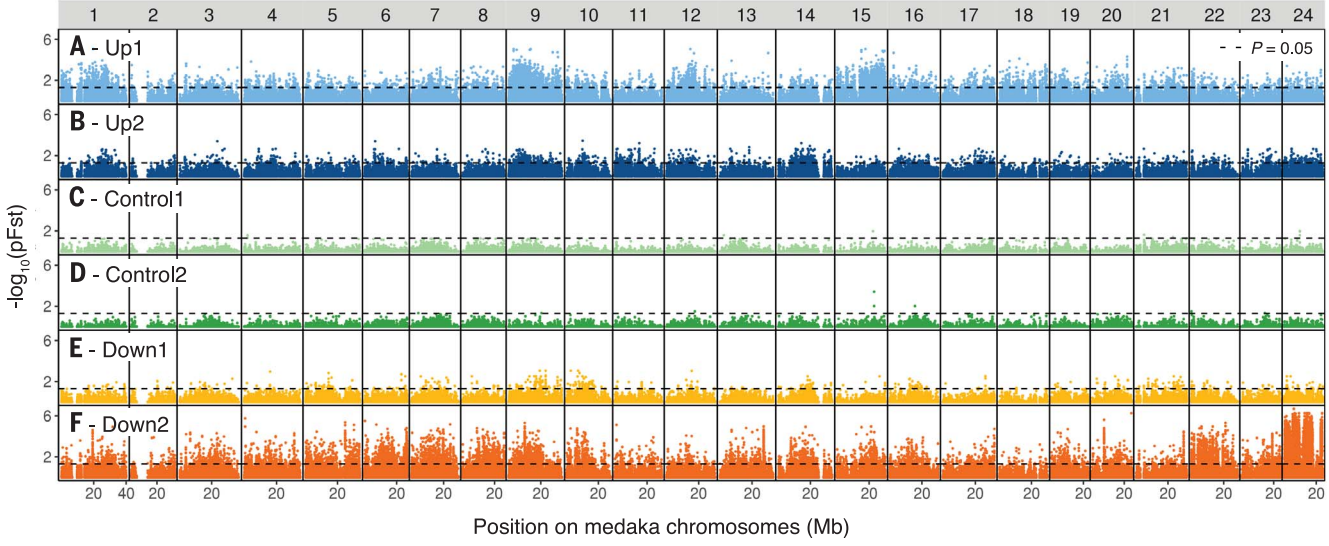

Fig. 3. Genomic distribution of allele frequency changes in each population. Log_{10}-transformed FDR-corrected *P* values for allele frequency change between generations 0 and 5 in the six experimental populations (**A** to **F**) at each SNP ordered along the 24 medaka chromosomes.

However, the four populations subjected to size selection (two up-selected and two down-selected) all suffered a significantly greater loss of diversity (23 to 27% loss of polymorphic sites and 7 to 9% loss of nucleotide diversity) than either of the two control lines (17 to 20% of polymorphic sites and 5% of nucleotide diversity, $P \leq 0.028$, one-tailed *t* test) (table S1). Using a likelihood ratio test that accounts for genotype uncertainty given our low-coverage data, we also observed a greater number of significant allele frequency shifts in the selected lines (529 to 19,258 SNPs) compared with the control lines (nine SNPs in each) (table S1) despite identical sequencing effort. Thus, the size-selection treatments consistently led to accelerated rates of genomic change.

Higher levels of relatedness (fig. S2) and fewer distinct mitochondrial haplotypes in the selected lines (table S1) suggest that this acceleration was partially caused by a selection-induced reduction in effective population sizes, which in turn increased genetic drift across the genome. However, drift will cause random fluctuations in allele frequencies, whereas we expect selection to change allele frequencies at genes affecting the targeted phenotype in the same direction under identical harvest regimes and in opposite directions under opposing harvest regimes. We used the statistic diffStat (*19*) to quantify parallel divergence as the minimum allele frequency difference in the same direction between up- and down-selected replicates. Simulations under a neutral scenario showed, on average, 4952 SNPs with diffStat > 0.3, whereas our data show 10,523 (Fig. 2A and fig. S3), suggesting that size selection caused consistent patterns of parallel divergence across more SNPs than what is expected by chance.

SNPs with diffStat values in the top 1% (greatest parallel allele frequency divergence between selection regimes, n = 23,648 SNPs) cumulatively show a clear correlation with individual body length across populations ($P < 10^{-6}$, linear mixed-effects model) (Fig. 2B), supporting their involvement in a polygenic response to selection. A subset of the same top 1% diffStat SNPs also exhibits highly elevated levels of differentiation in allele frequencies among the four wild populations (1596 with geographic F_{ST} > 0.25 and 357 with F_{ST} > 0.5 against an exome-wide

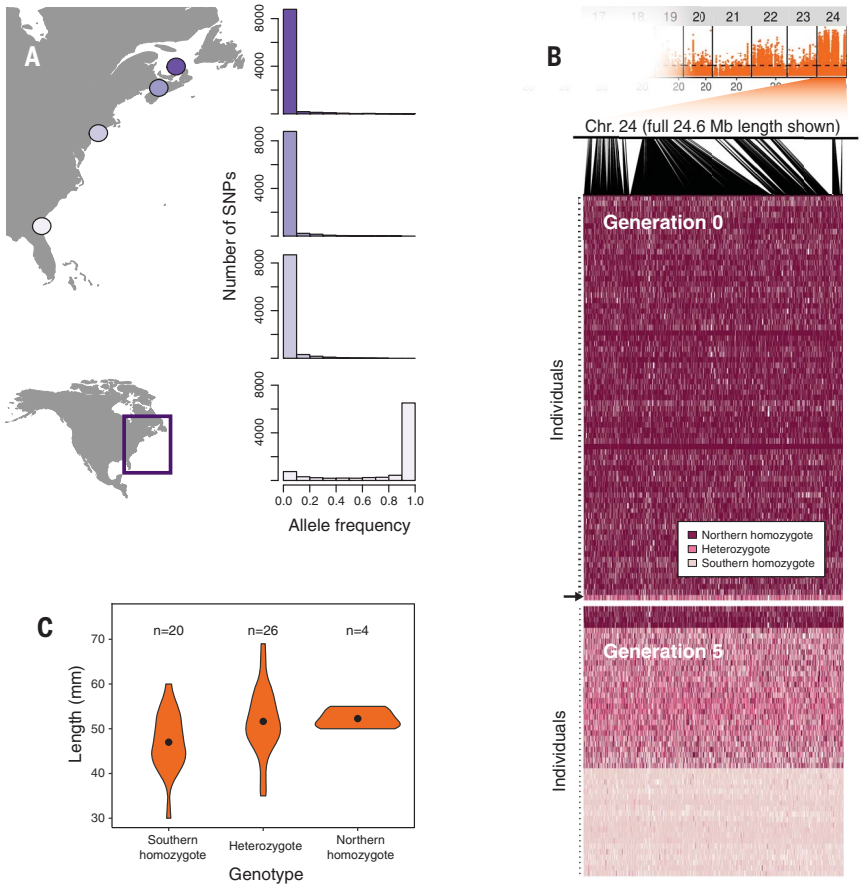

Fig. 4. Expansive linkage disequilibrium block on chromosome 24 shows fixed differences along the geographic cline and correlation to body length. (**A**) Histograms of the minor allele frequency distributions in four wild Atlantic silverside populations for the 9348 chromosome 24 SNPs that changed significantly in the Down2 population. (**B**) The most likely genotype at the subset of these SNPs (columns) with geographic F_{ST} > 0.9 (n = 5447 SNPs) for each individual (rows) in generation 0 (top heatmap) and in Down2 generation 5 (bottom heatmap). The single heterozygous individual in generation 0 is highlighted with an arrow. (**C**) Length distributions among individuals carrying either two, one, or zero copies of the southern chromosome 24 haplotype in the Down2 population in generation 5. Chr, chromosome.

median F_{ST} of 0.02). For the majority (83.2%) of the 357 SNPs that show the greatest geographic structure, the allele that is most common in the north (where short summers select for fast growth) became more frequent in the up-selected (fast-growing) experimental lines. Conversely, the northern allele decreased in frequency in the down-selected (slow-growing) lines for 89.9% of these SNPs (Fig. 2C). The pronounced bias in directionality of change suggests a shared genomic basis for growth rate divergence in the experiment and in the wild, implying that the rapid response to selection tapped into a reservoir of standing genetic diversity across hundreds of unlinked (fig. S4) variants maintained by long-term clines in natural selection. Functional enrichment analysis revealed significant overrepresentation [false discovery rate (FDR)–corrected P < 0.05] of these variants in 11 biological process categories, including biomineral tissue development, notochord morphogenesis, bone mineralization, and regulation of heart rate, all of which are possibly linked to growth (table S3).

However, our initial focus on parallelism revealed only part of the response to selection. Of SNPs that changed the most across generations [in the top 1% of temporal F_{ST} (generations 0 to 5) for each population], only 0.9% are the same between the two down-selected replicates, and only 2.3% are the same between the two up-selected replicates (fig. S5). Alignment to the medaka (*Oryzias latipes*) genome further indicates that SNPs with significant allele frequency changes are concentrated on different sets of chromosomes in the four different size-selected lines, indicating highly idiosyncratic responses (Fig. 3).

The most extreme change occurred in a block of 9348 SNPs on chromosome 24 that shifted from an initial frequency of <0.05 to ~0.6 in generation 5 in only one of the two down-selected populations ("Down2" in Fig. 3F). Almost all of these SNPs, held together in strong linkage disequilibrium (fig. S6), are either fixed or nearly fixed for opposite alleles between wild silverside populations in the north and south of the distribution range (Fig. 4A), indicating that this haplotype block has been under strong divergent selection across the latitudinal growth rate cline in the wild. The region showed no recombination over the course of the experiment (Fig. 4B and fig. S7B) yet may span much of the chromosome if synteny is conserved between medaka and the Atlantic silverside (fig. S7B). It stretches across 415 genes that show overrepresentation of four biological process categories relating to muscle contraction and calcium sequestration (table S4). Furthermore, these genes have 7% more nonsynonymous variants than the exome-wide proportion (significant enrichment, χ^2 = 49.49, df = 2, $P < 10^{-6}$), suggesting long-standing impacts of natural selection on the chromosomal variants.

Consistent with phenotypic patterns, it was the southern (naturally slow-growing) linked haplotype that increased in Down2, and it correlated negatively with individual body length (linear mixed-effects model P = 0.038) (Fig. 4C), explaining 7.9% of the phenotypic variation in Down2. Its frequency also reverted back toward initial levels after size selection had been relaxed in a five-generation continuation of the experiment (fig. S7), mirroring a phenotypic reversal to faster growth rates in the down-selected lines (*20*).

The much greater number of significant allele frequency shifts than expected under drift alone (fig. S8) followed by the reversal and coupled with the association with both growth divergence patterns in the wild and individual length in the experiment indicate that chromosome 24 played an important role in the response to selection in the Down2 population. Yet, we do not observe elevated allele frequency change in this genomic region in any of the other selected lines, including Down1 (Fig. 3). This may be because the experiment was established with fish from the middle of the species range, where the southern haplotype on chromosome 24 is rare (only one copy of 150 sampled haplotypes in generation 0) (Fig. 4). Thus, the southern haplotype may have been absent or lost early in the experiment in most populations, leaving little opportunity for selection to act on it. Nonetheless, the similar phenotypic responses to size selection across other replicates that did not have this haplotype available (Fig. 1) indicate that alternative genomic pathways must have been involved.

Similarly, in one of the up-selected populations (Up1), change is concentrated on chromosomes 1, 9, and 15 (Fig. 3A), coinciding with elevated linkage disequilibrium in these regions and strong drops in genetic diversity, indicating selective sweeps (fig. S9). More than 39% of SNPs in the top 1% of temporal F_{ST} (between generations 0 and 5) and more than 66% of SNPs in the top 0.1% map to these three chromosomes. The SNPs in the top 0.1% also cumulatively show a significant correlation with individual body length

(linear mixed-effect model, $P = 0.016$) (fig. S9C) and are enriched for nonsynonymous SNPs ($\chi^2 = 13.26$, df = 2, $P = 0.0013$) (fig. S10), supporting their functional role. The other up-selected replicate (Up2) shows small shifts on chromosomes 1 and 9 but not on 15 (Fig. 3B).

Taken together, our results thus show three major patterns. First, size-selective fishing caused greater loss of genetic diversity compared with size-independent fishing. Findings of reduced genetic diversity in wild, overharvested fish populations (*6*) have generally been interpreted as consequences of population bottlenecks. Our results suggest that fishery-induced selection may also have contributed.

Second, we see parallel allele frequency shifts among selection lines in hundreds of unlinked genes associated with growth variation in the wild. Such repeatable polygenic responses across many loci follow classic quantitative genetic predictions about the effects of selection on complex traits based on preexisting genetic variation (*11*) and suggest that natural variation maintained across environmental mosaics facilitates rapid responses to anthropogenic selection.

Third, in contrast, we also observe idiosyncratic signatures of strong, but highly nonparallel, selection on large blocks of tightly linked genes in some replicates. Linked gene complexes play an important role for local adaptation in other marine species (*21*, *22*), and multiple well-known cases of rapid adaptation have been attributed to large allele frequency shifts at one locus or a few key loci (*23*–*25*). This contrasts with systems in which adaptation is mediated exclusively by small shifts across many loci (*26*, *27*). We see a combination with similar phenotypic responses to selection (Fig. 1) underpinned by parallel polygenic shifts across all populations but large changes in linked genetic variation in only some of them.

The juxtaposition of these different modalities of genome evolution and the distinct evolutionary trajectories in each of our replicates illustrate how observations at the phenotypic level may provide impressions of parallelism even when part of the underlying genomic shifts are highly divergent. Although this pattern is sometimes found among wild populations (*28*), it counters previous reports of consistent genomic responses to replicated experimental evolution from standing variation in outbred sexual organisms (*13*, *29*).

Although the selection intensity used in this study was somewhat higher than in most real fisheries, and exact responses to selection always are influenced by the particular experimental design, our results clearly indicate that the genomic underpinnings of fishery-induced evolution, which have been invisible to us until now, are not predictable from phenotypic patterns alone. They also show that fishing may rapidly cause genomic changes that are comparable to differences between geographically distinct populations in nature. These findings likely hold true for many other species that, like the Atlantic silverside, harbor a diverse reservoir of adaptive standing genetic variation (*30*), enabling multiple pathways to the same rapid evolutionary response. We now have the capability to reveal and monitor such responses at the genome level, allowing fisheries and wildlife managers to more comprehensively assess human impacts, improving our understanding of the speed, consequences, and reversibility of complex adaptations as we continue to sculpt the evolutionary trajectories of the species around us.

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ACKNOWLEDGMENTS

We thank S. Kryazhimskiy for advice on library preparation, D. Pearce for facilitating sample processing, B. Sheets for assistance in the laboratory, R. Waples for helpful discussions, and the Therkildsen laboratory at Cornell University and four anonymous reviewers for valuable comments that helped improve the manuscript. **Funding:** This work was funded by National Science Foundation grants to S.R.P. (OCE-1434325) and N.O.T. (OCE-1756316), a Villum Foundation (Denmark) postdoctoral fellowship to N.O.T., and Cornell University. **Author contributions:** N.O.T. and S.R.P. conceived of and designed the study; N.O.T. generated the data; N.O.T. and A.P.W. analyzed the data with input from S.R.P.; S.B.M. and D.O.C. conducted the original experiment; H.B. recovered the samples and participated in the study design; and N.O.T., A.P.W., and S.R.P. wrote the manuscript with input from all authors. All authors approved the manuscript before submission. **Competing interests:** None declared. **Data and materials availability:** The genomic sequence data are archived in the NCBI Short Read Archive under BioProject ID PRJNA376564.

SUPPLEMENTARY MATERIALS

science.sciencemag.org/content/365/6452/487/suppl/DC1
Materials and Methods
Figs. S1 to S10
Tables S1 to S5
References (*31*–*65*)

18 January 2019; accepted 7 June 2019
10.1126/science.aaw7271

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Science **365** (6452), 487-490.
DOI: 10.1126/science.aaw7271

Parallel and idiosyncratic fish adaptation

Fish populations respond rapidly to fishing pressure. Within a handful of generations, marked phenotypic change can occur—often to smaller body sizes, because it is the big fish that are usually extracted. Therkildsen *et al.* examined wild ancestor fish lineages and found that polygenic mechanisms underpin this rapid evolutionary capacity (see the Perspective by Jørgensen and Enberg). Phenotypic change happened in two ways: first, by multiple small parallel changes in hundreds of unlinked genes associated with growth variation in the wild, and second, by shifts in large blocks of linked genes, causing large allele frequency changes at some loci.

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