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Report

Parallel Selection Mapping Using Artificially Selected Mice Reveals Body Weight Control Loci

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Summary

Understanding how polygenic traits evolve under selection is an unsolved problem [1], because challenges exist for identifying genes underlying a complex trait and understanding how multilocus selection operates in the genome. Here we study polygenic response to selection using artificial selection experiments. Inbred strains from seven independent long-term selection experiments for extreme mouse body weight ("high" lines weigh 42–77 g versus 16–40 g in "control" lines) [2] were genotyped at 527,572 SNPs to identify loci controlling body weight. We identified 67 parallel selected regions (PSRs) where high lines share variants rarely found among the controls. By comparing allele frequencies in one selection experiment [2–4] against its unselected control, we found classical selective sweeps centered on the PSRs. We present evidence supporting two G protein-coupled receptors *GPR133* and *Prlhr* as positional candidates controlling body weight. Artificial selection may mimic natural selection in the wild: compared to control loci, we detected reduced heterozygosity in PSRs in unusually large wild mice on islands. Many PSRs overlap loci associated with human height variation [5], possibly through evolutionary conserved functional pathways. Our data suggest that parallel selection on complex traits may evoke parallel responses at many genes involved in diverse but relevant pathways.

Results

Many fitness-related phenotypes, including numerous common human diseases, are genetically complex. As such, uncovering the genetic underpinnings of complex traits is a key goal in medicine, agriculture, and evolutionary biology [6]. Although finding the causal genes for simple Mendelian traits is now relatively straightforward [7], gene mapping for complex or quantitative traits remains difficult [1]. This is primarily because complex trait variation is caused by numerous quantitative trait loci (QTL). Complex traits are also particularly relevant for evolutionary adaptations, because most traits under selection are quantitative—therefore polygenic—in nature. However, in most models of natural adaptation, little is known about the target and the direction of selection, not to mention the genes, hence it is important to characterize the genomic response to selection in a well-defined system with a known target trait.

Body weight is an archetypal complex trait in mice [8–10] and was studied by mapping crosses, recombinant inbred lines, and long-term selection lines [9, 10]. But despite decades of intensive study, a fine-grained understanding of the genes underlying growth and/or body weight remains elusive. Body weight QTLs have been found on all 20 mouse chromosomes, many with intervals spanning entire arms [9]. Concerted positional cloning efforts have narrowed only a few of these QTLs to genes (e.g., *Glypican-3* and *PAPPA-2* [8, 11]; cf. spontaneous mutations *hg*/*Socs2*, *ob*/*Leptin* [12, 13]). Increased body weight in house mice has been observed repeatedly and independently, both under long-term artificial selection in the laboratory (Figures 1A–1C; see also Table S1 available online) and in natural populations in the wild [14]. Because most of these mice are primarily derived from the western house mouse *M. m. domesticus* and share recent genetic ancestry [15], some part of the response to selection is likely to have a shared allelic basis [16]. To advance our understanding of complex trait genetics and evolution, we applied "parallel selection mapping" [17] to identify this component of shared loci underlying parallel increase in body weight across multiple long-term artificial selection experiments in mice.

Long-Term Selection in Mice and Parallel Association Mapping

Long-term artificial selection has been popular in quantitative genetics since the 1930s, with mouse selection colonies repeatedly founded in the 1970s in Germany (Berlin, Dummerstorf, Munich), the United Kingdom (Edinburgh, Roslin) and the United States (Davis, CA and Raleigh, NC; Figure 1A; summarized in [2, 18]). Beyond their differences (stock composition, effective population size, trait, strength and timing of selection; Table S1), all these experiments produced significantly heavier and larger mice than unselected controls or selected small mice (body weight at 70 days [d]: high body-weight-selected lines: 42–77 g; control or low body-weight-selected lines: 16–40 g [2]; Figures 1B and 1C), reaching as much as 240% divergence in body weight in the Dummerstorf experiment (averaged selection intensity i = 0.73 ± 0.17 SD) [3]. In 2001, Bünger and coworkers developed inbred lines from these stocks [2], forming the basis of our present genomic analysis.

To identify shared variation among the derived inbred high lines that differ from their paired control lines, we genotyped one to two individuals per line using the Affymetrix Mouse Diversity Array featuring 584,726 SNPs (n = 23 across 13 lines) [19]. Genome-wide hierarchical clustering groups sibling high and control lines from a given selection experiment, suggesting that on a genome-wide scale, high lines are not similar to each other (Figure S2), as expected given their independent origins (Table S1). To examine local genetic signals, we built 13-by-13 pairwise genetic distance matrices using 25-SNP windows (approximately 50 kb, well within typical haplotype block size found in outbred stocks [16]) sliding with 5-SNP increments across the genome. We applied a statistic called

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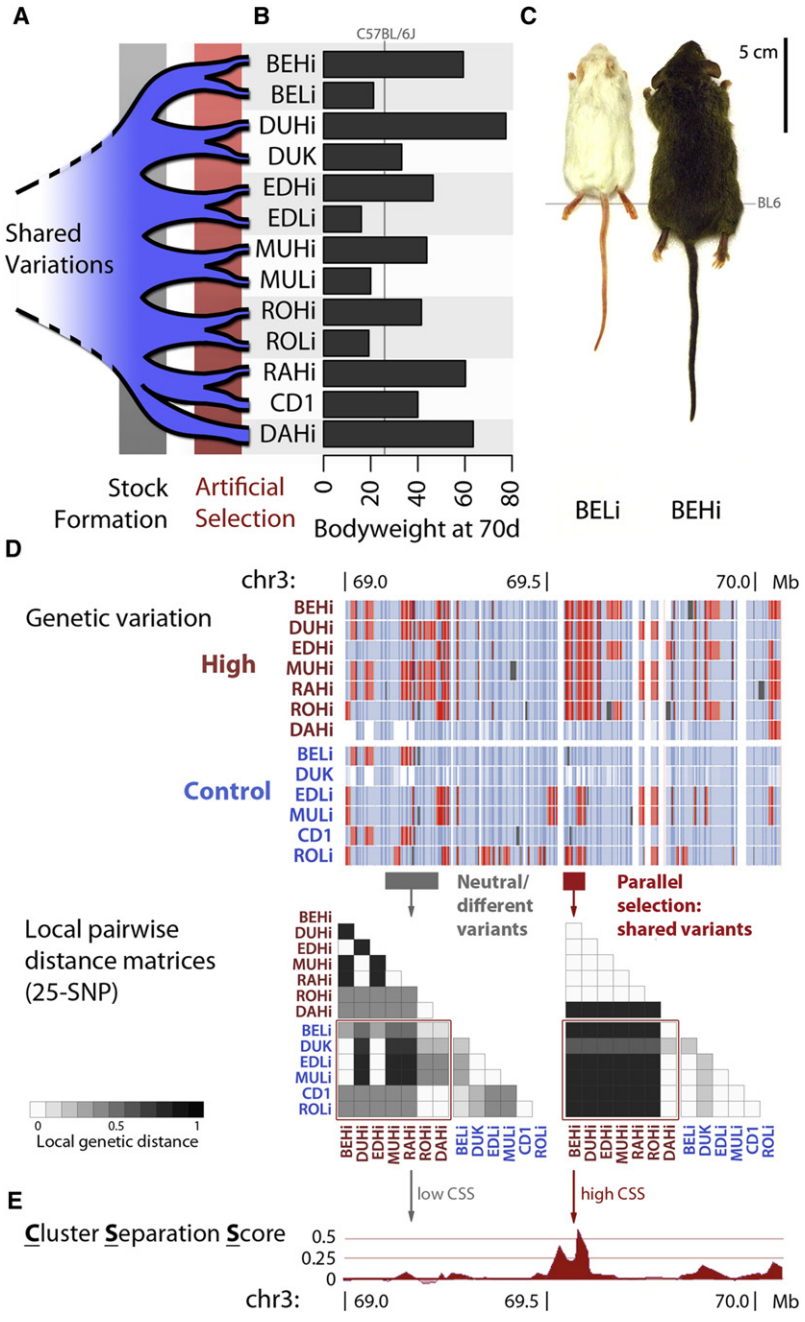

Figure 1. Parallel Selection Mapping Using Long-Term Selected Mouse Lines

(A) Recent derivation of laboratory mice makes all laboratory mice related and has led to many shared variations [16]. Artificial selection experiments on increased body weight have been conducted independently on mouse stocks presumably carrying common shared genetic variation.

(B) Body weight differences at 70 d among the different high body weight selected and their control lines (see Table S1 for information on these lines). Light gray bar indicates the body weight of the reference genome mouse line C57BL/6J, a common unselected laboratory line.

(C) Adult female BELi and BEHi mouse: 64 generations of selection on mice from a pet store has produced not only heavier but also larger BEHi mouse. Selection on 60 d lean and overall weight has also produced accompanying coat color differences. The average body length of the reference genome mouse line C57BL/6J is indicated by a gray line.

(D) High density genotyping was performed on these mouse lines, producing reference (C57BL/6J) homozygous genotypes (blue); alternate allele homozygous genotypes (red); heterozygous positions (white) or missing data (gray). We generated 13-by-13 pairwise distance matrices for 25-SNP sliding windows across the genome. For each window of the genome, CSS (red plot) was calculated. Examples are shown for two windows of neutral and/or different variation resulting in low CSS (left matrix) and parallel shared variation resulting in high CSS (right matrix) respectively.

(E) Allele sharing between high lines due to parallel selection results in excessive divergence between high and control mice relative to divergence observed within high mice and within control mice (right matrix, red plot). See also Figure S1.

"cluster separation score" (CSS) to quantify genetic distance between a priori groupings of lines (here between-group high vs. control distances) after accounting for within-group genetic distances [20] (Figure 1D; see Supplemental Experimental Procedures). Hence, high CSS reflects parallel divergence between high and control lines, most likely due to local sharing of standing genetic variation, i.e., haplotypes, in individual selection experiments despite their independent derivations (Figures 1A and 1D).

Genome wide, CSS is centered close to 0 (indicating little excess genetic differentiation between high and control lines) with a long tail indicating genomic windows with high divergence between high and control lines that has occurred in a majority of selection experiments (Figure 2A; Figure S3). After applying a significance cutoff, we could identify 67 parallel selected regions (PSRs) in the genome with a false discovery rate of less than 1% (FDR $\leq$ 0.01; Figure S3B; by coverage: 37.9 Mb [1.4% of the genome]; by gene: 525 [2.7% of all genes]; Table S3; Supplemental Experimental Procedures). PSRs range mostly between 50 kb to 1 Mb in size (Figure 2B), corresponding to sub-cM resolution and often identifying a single gene or subsegments of a gene, including several non-coding regions. The high resolution observed in our approach stands in contrast to the megabase-spanning confidence intervals found in most QTL mapping studies (Figure 2B; reviewed in [9]). Genome wide, PSRs are found as dispersed clusters: they occur on many chromosomes but with significant intrachromosomal clustering (Figure 2A; $p < 5 \times 10^{-6}$, Mann-Whitney U test, $H_0 \leq 0$, W = 17,259,563). Many PSR clusters are contained within larger QTL intervals (Figure 2A). Some may correspond to linked loci that combine to function as a large-effect QTL [6, 11].

Multilocus Signature of Selection Identified at PSRs

To explore how multilocus selection operates in the genome and characterizes the molecular signatures of selection around the 67 PSRs, we genotyped multiple individuals sampled from each of two time-points in the longest ongoing bodyweight-selection experiment from Dummerstorf, Germany [2]

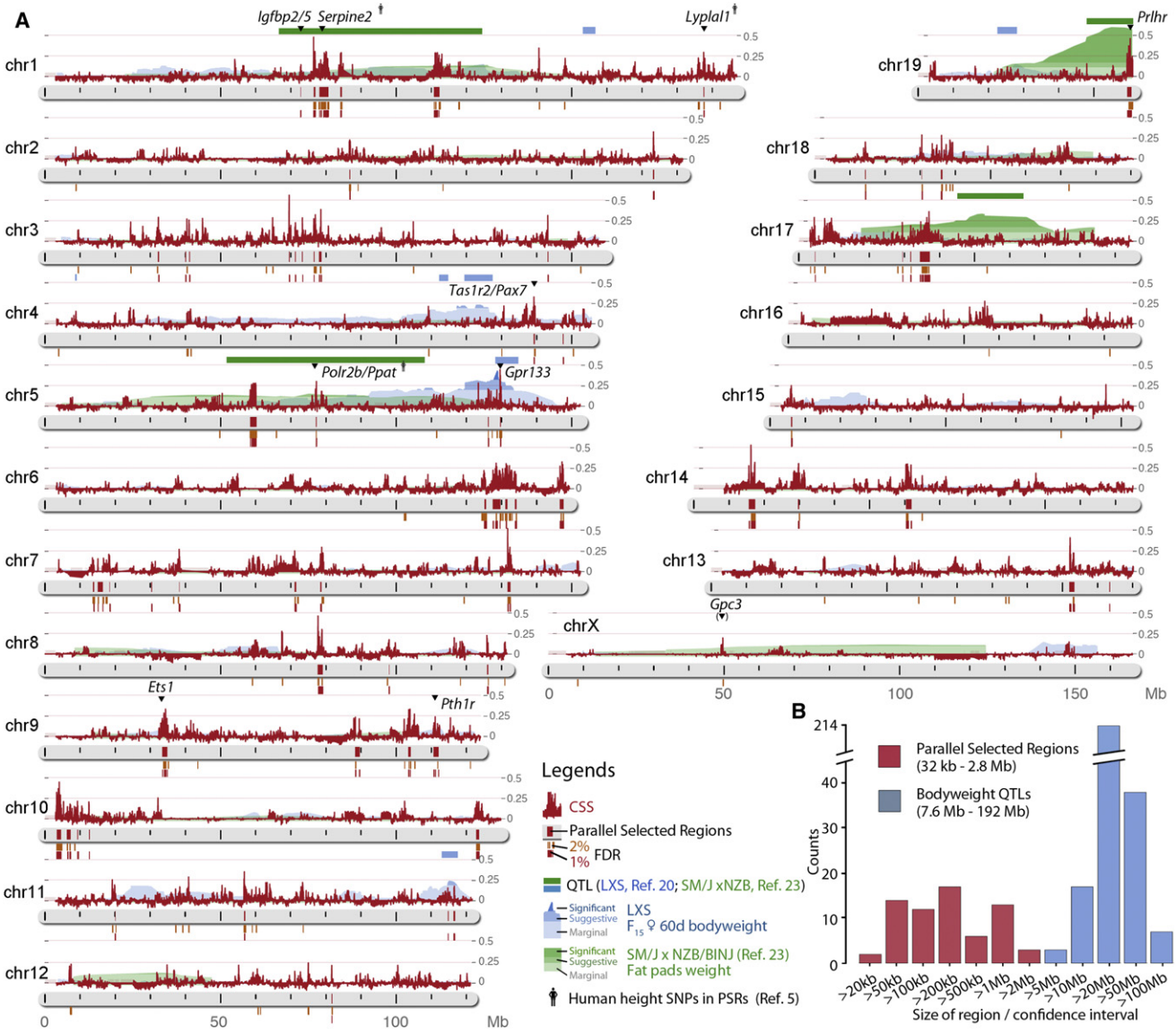

Figure 2. Parallel Selected Regions Are Detected on Many Chromosomes

(A) Chromosomes are represented as gray bars. CSS (red graph) is plotted above each chromosome, and PSRs are represented by red segments on the chromosome bar. Individual windows detected with 2% (orange, higher ticks) or 1% (red lower ticks) FDR are shown as tick marks beneath the chromosome. PSRs are found on almost all mouse chromosomes. Their genomic distribution is nonrandom and shows significant clustering. The X chromosome contains no PSR, possibly due to array design considerations (see Supplemental Experimental Procedures and [19]). Published QTL intervals are indicated as colored horizontal bars above the chromosome (♀ 60 d body weight [20]: blue, and obesity and body weight *Obwq5* [23]: green) and continuous plots for LOD support (darker colored shading indicates higher genome-wide significance). Candidate genes for a subset of PSRs are indicated with black text and arrowheads. (B) PSRs have higher resolution than QTL confidence intervals. There is no overlap in the binned size distribution of the 67 PSRs detected in the current study (red bars) and 279 mouse body weight QTL intervals detected in other studies (summarized by http://www.obesitygenes.org/). See also Figure S2.

(generations 80 and 154, n = 6 each) to compare against their unselected control (randomly mated for >150 generations, n = 10). Using variable SNPs in the combined sample, we sought to detect classical signatures of selection (decreased heterozygosity, excessive rare alleles, extended haplotypes, and increased differentiation) between the selected and their control populations at PSRs compared to background regions. For all four statistics, we observed significant deviations in PSRs compared to background loci (Figures 3A–3D; generation 80 PSRs versus background, all summary statistics [Mann-Whitney U tests]: $p \leq 1 \times 10^{-8}$; generation 154 PSRs versus background, all statistics: $p \leq 1 \times 10^{-15}$; control PSRs versus background, all tests: n.s.). These signals became stronger from generation 80 to 154 (Figures 3A–3D), suggesting that ongoing parallel selective sweeps due to strong multilocus selection produced the observed pattern of allele-sharing across independent selection experiments.

Genes and Pathways Consistently Involved in Increased Body Weight

For these regions to be selected in parallel across independent body weight selection lines, we would expect PSRs to be enriched for growth and body weight control gene functions (see Supplemental Experimental Procedures for gene coverage

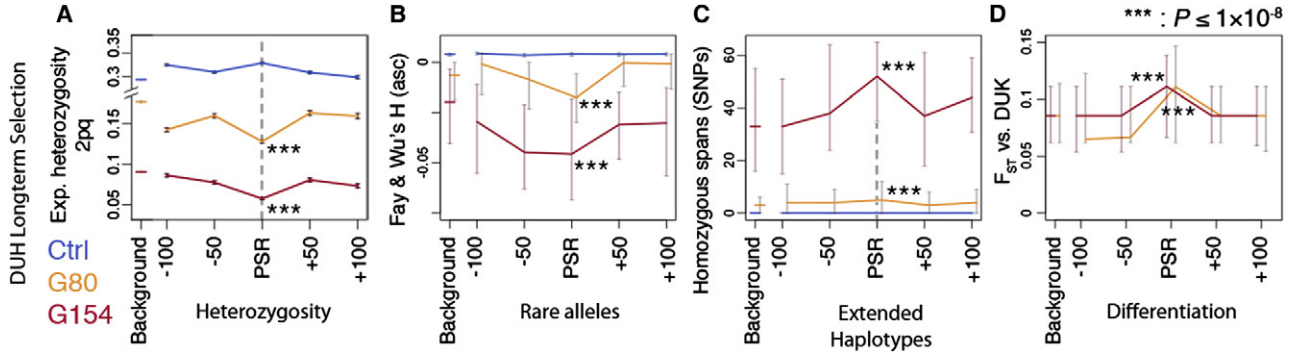

Figure 3. Evidence for Signature of Selection around PSRs

(A) Decreased diversity (expected heterozygosity).

(B) Excessive proportion of rare alleles (Fay & Wu's H).

(C) Long extended haplotypes (homozygous span, see Supplemental Experimental Procedures).

(D) Increased differentiation located within the PSRs (F_{ST}). Each panel shows a population genetic summary statistic from the Dummerstorf long-term selection experiment, comparing the 80th generation (G80/yellow) and 154th generation (G154/red) selected mice against a control nonselected DUK cohort (Ctrl/blue). Statistics are shown for SNPs that fall within the PSRs and flanking 50-SNP bins (four bins, from −100 to +100) as well "background loci" (based on all other non-PSR SNPs). Error bars for heterozygosity indicate ± SEM. Error bars in the remaining plots indicate the range from the 40th to the 60th quantile. In all four statistics, the signals from within the PSRs deviate significantly from background patterns in the G80 and G154 selected mice (all $p \leq 1 \times 10^{-8}$, Mann-Whitney U test) but nonsignificantly in control nonselected mice.

definition). We categorized gene functions using knockout phenotype categories according to Mouse Genome Informatics or Kyoto Encyclopedia of Genes and Genomes (KEGG) assigned pathway and tested for enriched categories. Among the phenotype categories, we found that PSR-associated gene knockouts are significantly more likely to result in "abnormal birth body size" ($p = 0.021$, permutation test; Table S3). Among the KEGG pathways, we observed a significant enrichment of PSR-associated genes in body weight regulating pathways: the adipocytokine signaling pathway ($p \leq 0.001$, permutation test) and the related insulin, mammalian target of rapamycin (mTOR), and phosphatidylinositol pathways (energy uptake and growth, e.g., PSR-associated genes *Pik3r1*, *Pik3ca*, and *Mtor*; all $p \leq 0.025$, permutation test; Table S3). In addition, multiple pathways activated by G protein-coupled receptors (GPCRs), such as neuroactive ligand-receptor interaction and taste transduction functions (including several bitter taste and olfactory receptor clusters) also showed enrichment (both $p \leq 0.04$, permutation test; Table S3).

To assess whether gene expression is affected in corresponding pathways, we assayed liver and muscle gene expression from 9 out of the 13 mouse lines using Agilent microarrays. We found that genes showing significant expression differences between the high and the control lines at the multiple testing-corrected $p \leq 0.05$ level were enriched for pathways affecting body weight including genes in the adipocytokine signaling pathway (liver, hypergeometric test, $p \leq 0.0018$; Table S3), cardiac muscle contraction, and insulin signaling pathway (muscle, hypergeometric test, $p \leq 0.0013$ and $p \leq 0.018$ respectively; Table S3).

PSRs Predict Body Weight in Independent Mouse Panels

If reuse of standing genetic variation across independent selection experiments is common enough to allow parallel selection mapping, we would expect to also find PSRs to fall within body weight QTLs in otherwise unrelated mouse crosses. To test this, we obtained data from the biggest publicly available recombinant inbred line (RIL) panel LXS (ILS-by-ISS cross), which consists of 77 densely genotyped lines with highly replicated body weight measurements at multiple time points in both sexes. Two lines of evidence suggest that the PSRs correspond to LXS QTLs. First, the PSRs were significantly colocalized with published LXS QTLs (minimal distance between PSRs and the peak markers of significant [$p \leq 0.05$] and suggestive QTLs [$p \leq 0.63$] in Bennett et al. [20]; PSRs versus 1,000 permuted data sets, $p < 1 \times 10^{-15}$, Mann-Whitney U test, $H_0 \leq 0$, $W = 186{,}972.5$). Second, using only genotypes from 78 markers located inside or immediately adjacent to the 67 PSRs (out of a total of 2,065 genome-wide markers that were genotyped in individuals from the 77 LXS lines), a linear model of body weight in LXS individuals could predict between 51% and 94% of the variation in the phenotype (Table S4). These markers likely capture much of the phenotypic variation and out-perform randomly-chosen marker sets. We conclude that a shared genetic basis to phenotypic variation is likely to be common across laboratory experiments.

Positional Candidates for Major Loci Controlling Body Weight

We found that our high-resolution single-gene PSRs often overlap the large intervals of major LXS body weight QTLs (Figure 2A). One LXS QTL on distal chromosome 5 affects body weight during all growth phases in both sexes (e.g., female 60 d QTL, log of odds [LOD]: 5.53; peak marker rs13478521, confidence interval: 119–133 Mb; 1.015 g additive effect; Figures 2 and 4A–4E). We identified two small PSRs (63 kb and 141 kb, respectively) within the 14 Mb confidence interval, with one (Chr5:129,606,105–129,747,180) showing clear differentiation of two haplotypes and containing a single gene, the G protein-coupled receptor 133 (*GPR133*, Figures 4A–4C). *GPR133* is expressed during mouse embryonic development in the thymus primordium and the adrenal glands [21], key centers for hormonal regulation of body weight. Variants at *GPR133* in humans have also been shown to explain 0.95 cm in height in three different European populations [22].

Because strong selection may preferentially act on variants with strong phenotype effect, we examined another QTL, *Obwq5*, which controls the largest multilocus interaction

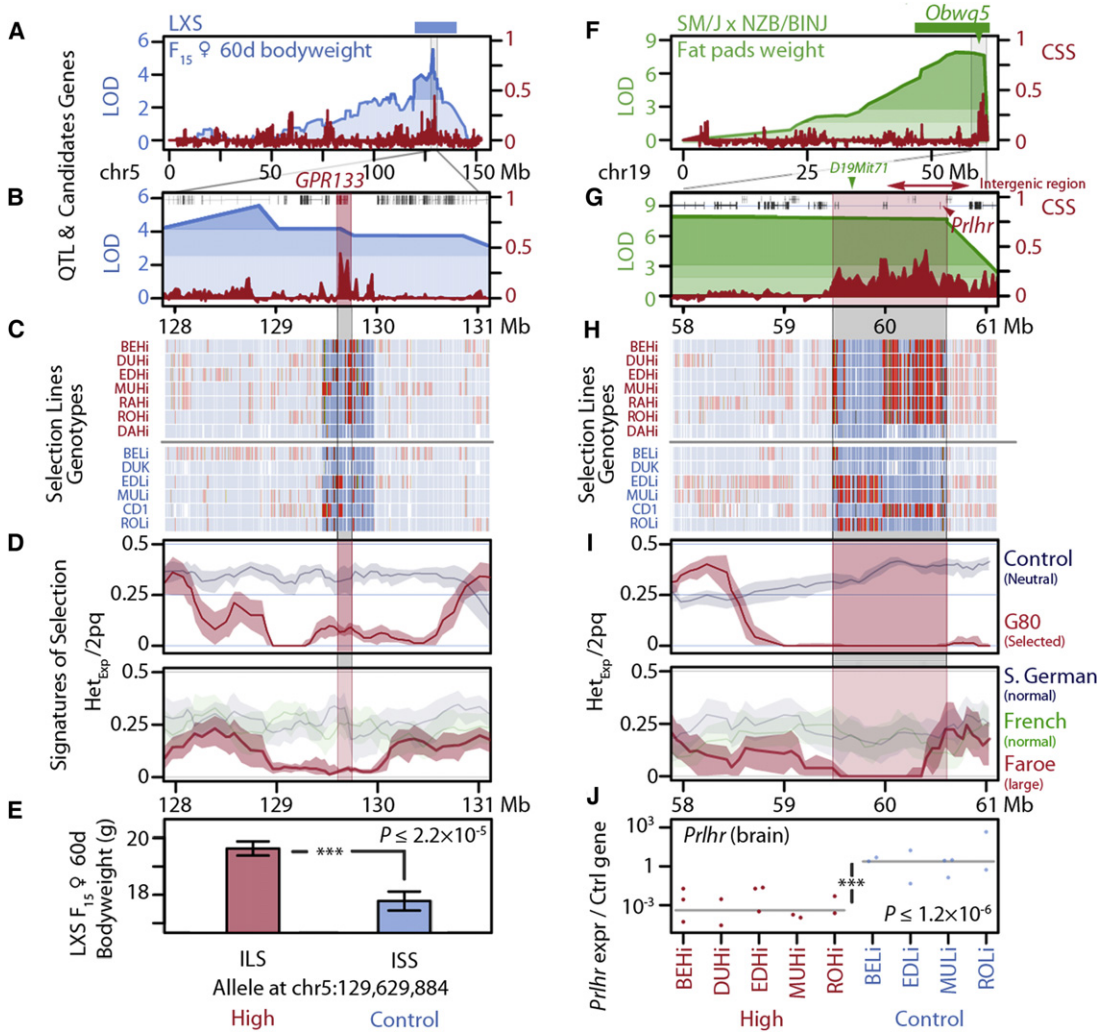

Figure 4. Body Weight PSRs Identify Candidate Genes for Body Weight Control

(A) A genome-wide significant LXS body weight QTL is located on Chr5 at approximately 130 Mb (blue LOD plot) [20].

(F) A second QTL for obesity *Obwq5* (based on a composite of lean body weight, and inguinal, gonadal, retroperitoneal, and mesenteric fat pads) [23] is located at the distal end of Chr19 at approximately 59 Mb (green LOD plot, peak marker *D19Mit71* is indicated by the arrowhead). Both QTLs fall within confidence intervals of approximately 14 Mb in size (indicated by horizontal blue [A] and green [F] bars above the plots) [20, 23]. CSS (red) suggests that genes located within small PSRs may contribute to each QTL.

(B and G) Lower plots show the local region near the LOD and CSS peak. A small PSR (red shading) overlaps (B) a single *G protein-coupled receptor 133* (*GPR133*) or (G) seven genes (the four hypothetical genes are plotted in lighter color above known genes), with the peak CSS signal in the intergenic region flanking another G protein-coupled receptor, *prolactin releasing hormone receptor* (*Prlhr/GPR10;* red arrow).

(C and H) The genetic variation among the selection experiments at both *GPR133* and *Prlhr* show that high lines share a similar haplotype, whereas different haplotypes are found among the control lines in the PSR (the reference C57BL/6 allele among control lines colored blue, alternate allele colored red, heterozygous sites white).

(D and I) Selective sweeps around both candidate genes *GPR133* and *Prlhr* are apparent as reduction in heterozygosity in the selected 80th generation (red) of the Dummerstorf selection experiment. In contrast, the unselected control (blue) population does not show similar reduction in the same region. Within the PSRs for both regions, there is no overlap in their 95% confidence intervals (shading, based on bootstrap resampling. See Supplemental Experimental Procedures). Selective sweeps coincident with the PSRs are found among the large Faroe (red) population, but heterozygosity near normal levels is retained in the French (green) and S. German (blue) populations.

(E) In the LXS mapping panel of recombinant inbred lines, those lines carrying the high body weight allele identified in the present study as a PSR body weight locus in independent long-term selection lines (ILS, red) are significantly heavier than those carrying the alternate allele (blue). This corresponds to an additive effect of 0.93 g per high/ILS allele. Error bars indicate ± SEM.

(J) Consistent with the obese phenotype in *Prlhr* null mice, qPCR measurements from multiple individuals (dots) show a consistent and significant reduction of *Prlhr* expression in high mice brain tissues than in control mice. None of the other six genes in the *Prlhr* PSR interval showed body weight knockout phenotypes. See also Figure S3.

network controlling both lean body weight and fat pad weights in the SM/J × NZB/BINJ cross [23]. It is located on distal Chr19 (44–52 cM, peak marker: *D19Mit71* at Chr19: 59.6 Mb; and is a composite of eight significant body weight QTLs including lean body weight and various fat pad weights with LOD from 4.4 to 9.5 [23]; Figures 2A and 4F–4J). *Obwq5* contains a high resolution PSR, which features another GPCR called prolactin releasing hormone receptor (*Prlhr/GPR10*). *Prlhr* is flanked by

a large intergenic region, in which the high and the control lines harbor nearly mutually exclusive alleles and which also corresponds to a selective sweep signature in the Dummerstorf time-series (Figures 4H and 4I). *Prlhr* is normally expressed in the hypothalamus [24], which coordinates appetite and metabolic rate and is responsive to insulin and blood glucose levels. By quantitative PCR we found that *Prlhr* is downregulated in the brain of high lines compared to control lines [analysis of variance (ANOVA), $F(1,19) = 48.76$, $p \leq 1.19 \times 10^{-6}$; Figure 4J]. Consistent with *Prlhr* downregulation, the NZB/BINJ allele at the *Obwq5* QTL acts recessively to confer higher weight [23]. Further, *Prlhr*-null mice exhibit hyperphagia, suffer from late-onset obesity, and become prediabetic [24]. Combined evidence from this and other knockout experiments of GPCRs (e.g., PSR-associated genes *Pthr1* [23], see review by [24]) suggest that GPCRs may feature prominently in a polygenic response to selection on body weight.

Beyond Artificial Selection: PSRs in Wild Mice and Human Evolution

Next, we asked whether the PSRs might also account for natural evolution of large body size in wild mice. In rodents, large body size (and higher body weight) has evolved repeatedly on islands around the world and may be selectively favored [14, 25, 26]. The Faroese and the now-extinct St. Kilda (Scotland) house mice have evolved within 1,000 years to be among the biggest wild mice in the world [27], including increased mandible size [mandible area, ANOVA, $F(1,158) = 46.79$, $p \leq 1.64 \times 10^{-10}$; Figure S3]. We observed lower heterozygosity in PSRs than the background levels in island mice from both the Faroese and St. Kilda populations, but not their normal-sized continental counterparts (all Mann-Whitney U test, $H_0 \leq 0$, heterozygosity of PSR SNPs [n = 4,350] versus non-PSR SNPs [n = 208,906] in each population; Faroe: $p \leq 8.5 \times 10^{-10}$; St. Kilda: $p \leq 0.0036$; continental populations: France, N. Germany, and S. Germany, all tests, n.s.; Figures 4D and 4I). This suggests that PSRs, identified under artificial selection for body weight, may recapitulate at least a subset of genes subject to natural selection in wild mouse populations evolving large body size. If enough such cases can be found, parallel selection mapping on natural large and matched normal continental mouse populations could potentially provide even higher mapping resolution due to the greater number of historical meioses accumulated in wild populations.

Having observed the connection between *GPR133* and human height, we asked whether this relationship applies generally. Taking the 180 human height loci in a human genome-wide association meta-analysis [5], we found their corresponding location in the mouse genome to be located closely to our PSRs (n = 154 loci, closest SNP-PSR distance, $p \leq 0.008$, permutation test). Out of 154 loci (defined as peak marker ± 1 Mb spans in the human genome, see [5]), 4 fall directly within PSRs and 16 (10.4%) fall within 1 Mb of a PSR, which is significantly more than expected (PSR ± 1 Mb coverage: 5.9% of genome, chi-square test, χ^2 [1, n = 154] = 5.6488, $p \leq 0.018$; Table S4). By inspecting the variations at these PSRs and mouse functional data, we propose likely alternatives to the ones based strictly on human data [5] (Table S5).

Discussion

Identification of genes underlying a complex trait is a key focus of community efforts in mouse genetics and remains a major challenge [28]. We have shown here that parallel selection mapping represents a novel approach to map complex traits. Our findings have both specialized and general relevance to complex trait genetics under selection. First, PSRs have superior resolution to QTL mapping. Many PSRs provide single-gene resolution, leading us to identify variants at *GPR133* and *Prlhr* as candidate genes within previously large QTL intervals. Where suitable conditions exist (e.g., replicated persistent selection on shared variants), we have demonstrated how parallel selection mapping can be a powerful general approach for rapid high-resolution gene mapping with minimal resequencing or genotyping costs. Second, our evidence for multilocus strong selective sweeps on standing variation (likely due to unusually strong truncation selection, e.g., 0.73 selection intensity in the Dummerstorf experiment [3]) contrasts with recent findings in human and *Drosophila*, which suggested that selection acting on standing variants may only rarely produce selective sweeps [29, 30]. On the other hand, all three studies agree that standing variation may play a key role enabling rapid (adaptive) response to changes in selection and environments. Such variations may be broadly relevant, as our wild mice results suggest that artificial selection may mimic natural adaptation. Deeper evolutionary conservation may also exist [6, 31], which could explain the connection between PSRs and human height loci. By identifying candidate genes and defining the genomic architecture for body weight in long-term artificial selection lines, we will help connect genes to a complex trait with medical, agricultural, and evolutionary implications.

Accession Numbers

Microarray expression data and genotyping data are available at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) under accession number GSE36452.

Supplemental Information

Supplemental Information includes three figures, five tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cub.2012.03.011.

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