

1 **Title:** Recent evolution in *Rattus norvegicus* is shaped by  
2 declining effective population size

3 **Running title:** Recent evolution in *R. norvegicus*

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24 *lus castaneus*

## Abstract

The brown rat, *Rattus norvegicus*, is both a notorious pest and a frequently used model in biomedical research. By analysing genome sequences of 12 wild-caught brown rats from their ancestral range in NE China, along with the sequence of a black rat, *R. rattus*, we investigate the selective and demographic forces shaping variation in the genome. We estimate that the recent effective population size ( $N_e$ ) of this species =  $1.24 \times 10^5$ , based on silent site diversity. We compare patterns of diversity in these genomes with patterns in multiple genome sequences of the house mouse (*Mus musculus castaneus*), which has a much larger  $N_e$ . This reveals an important role for variation in the strength of genetic drift in mammalian genome evolution. By a Pairwise Sequentially Markovian Coalescent (PSMC) analysis of demographic history, we infer that there has been a recent population size bottleneck in wild rats, which we date to approximately 20,000 years ago. Consistent with this, wild rat populations have experienced an increased flux of mildly deleterious mutations, which segregate at higher frequencies in protein-coding genes and conserved noncoding elements (CNEs). This leads to negative estimates of the rate of adaptive evolution ( $\alpha$ ) in proteins and CNEs, a result which we discuss in relation to the strongly positive estimates observed in wild house mice. As a consequence of the population bottleneck, wild rats also show a markedly slower decay of linkage disequilibrium with physical distance than wild house mice.

## Introduction

Comparative genomics became possible between human and mouse with the publication of the mouse genome (Mouse Genome Sequencing Consortium, 2002), leading to many important new findings, including estimates

1 of the fraction of conserved nucleotide sites, corroboration of downwardly  
2 revised estimates of protein-coding gene number, and the discovery of ul-  
3 traconserved non-coding elements (Bejerano et al., 2004). Genome sequenc-  
4 ing of single individuals naturally led to population genomics, pioneered in  
5 *Drosophila simulans* (Begun et al., 2007). This allows detailed inferences  
6 to be made concerning many important questions in evolutionary genetics,  
7 including the demographic history of populations (Li and Durbin, 2011), the  
8 nature and frequency of adaptive evolution (e.g., Hernandez et al., 2011; Sat-  
9 tath et al., 2011), and the causes of the correlation between recombination  
10 rate and neutral diversity (Cai et al., 2009).

11 Much population genetics has traditionally relied on comparing nucleotide  
12 polymorphism in one species to divergence from another. With decreas-  
13 ing sequencing costs, comparative population genomics - the comparison of  
14 multiple genome sequences from different species - has now become possi-  
15 ble. This allows reciprocal analysis of polymorphism *versus* divergence. For  
16 example, the McDonald-Kreitman test (McDonald and Kreitman, 1991) for  
17 estimating the relative extent of adaptive evolution, uses polymorphism in  
18 one species (A) and the divergence between two species (A and B). From the  
19 assumptions of the test, including a stable demography since the divergence  
20 between the two species, the reciprocal estimate should yield the same re-  
21 sult. A significant difference in the estimates could, therefore, indicate that  
22 an evolutionary important demographic change has occurred since the split.  
23 For such a reciprocal analysis to be biologically meaningful, the species need  
24 to be closely related, but not so closely related that they share a substantial  
25 fraction of nucleotide polymorphism that originated prior to the split be-  
26 tween the species. This condition is comfortably met in the case of our focal  
27 species, wild brown rats and mice, which are thought to have diverged at

1 least 12MYA (Benton and Donoghue, 2007), equating to at least 24 million  
2 generations.

3 One of the primary determinants of the efficacy of selection across the  
4 genome is the strength of genetic drift. The rate of drift is inversely propor-  
5 tional to the effective population size ( $N_e$ ), which represents the size of an  
6 ideal population that would display the observed amount of drift.  $N_e$  pre-  
7 dicts a number of fundamental properties of natural populations, including  
8 the amount of genetic variation ( $N_e \times$  mutation rate ( $\mu$ )), the rate at which  
9 linkage disequilibrium (LD) is broken down ( $N_e \times$  recombination rate ( $r$ ))  
10 and the strength of selection ( $N_e \times$  selection coefficient ( $s$ )). For example,  
11 theory predicts that if the scaled selection coefficient is below one ( $N_e s < 1$ )  
12 genetic drift will dominate over selection, rendering such mutations ‘effec-  
13 tively neutral’. The proportion of such mutations is therefore predicted to  
14 increase across the genome through demographic processes that reduce  $N_e$ ,  
15 such as fluctuating population size or bottlenecks. The interaction between  
16 genetic drift and selection can be manifest in a number of different ways,  
17 including an increase in the fraction of mutations in functional regions that  
18 behave as slightly deleterious or a lower rate of adaptive evolution.

19 In this paper, we compare a population genomic dataset of wild brown  
20 rats with a previously published dataset from wild house mice (Baines and  
21 Harr, 2007; Halligan et al., 2013) and investigate the differential effects of  
22 drift on the genomic signature of selection acting in protein-coding and con-  
23 served non-coding DNA in the genome. The effective population size in our  
24 focal population of wild house mice (*Mus musculus castaneus*) is nearly two  
25 orders of magnitude higher than recent  $N_e$  for human populations and sub-  
26 stantially higher than that of inbred lab strains (Salcedo et al., 2007; Baines  
27 and Harr, 2007; Phifer-Rixey et al., 2012). Halligan et al. (2013) inferred

1 that the protein-coding and conserved noncoding elements (CNE) evolved  
2 more rapidly than the neutral theory expectation, suggesting that there has  
3 been substantial genome-wide adaptation in proteins and CNEs of wild mice.  
4 Moreover, they found reductions in neutral diversity around protein-coding  
5 exons and CNEs, indicative of frequent selective sweeps and/or background  
6 selection. In contrast, despite a presumably large contemporary census pop-  
7 ulation size in wild brown rats (*Rattus norvegicus*), it has been estimated  
8 that their  $N_e$  is five-fold smaller than wild house mice (Ness et al., 2012).  
9 The difference in the  $N_e$  between mice and rats provides an opportunity to  
10 investigate the effects of genetic drift in the mammalian genome and the  
11 way in which selection and drift interact to shape patterns of diversity in  
12 the genome.

13 Using whole genome data from 12 rats collected from their ancestral  
14 range in NE China and a comparable dataset in wild house mice, we ask  
15 a number of questions (1) Does reduced  $N_e$  in rats lead to reduced efficacy  
16 of selection on new mutations affecting protein or CNE sequence? (2) How  
17 does the effect of hitchhiking differ between mice and rats and how does this  
18 compare between protein-coding exons and conserved non-coding elements?  
19 (3) Does reduced  $N_e$  in rats influence the extent of LD? (4) What can  
20 patterns of DNA polymorphism tell us about the recent demographic history  
21 of wild brown rats? We find strong evidence for a population bottleneck that  
22 has distorted the distribution of allele frequencies throughout the genome  
23 and altered patterns of LD in wild rats. We also find evidence for a higher  
24 frequencies of segregating deleterious mutations in wild rats, consistent with  
25 a reduction in the efficacy of purifying selection. However, neutral diversity  
26 reductions around protein-coding exons follow a virtually identical pattern in  
27 the two species, suggesting that selection has had similar effects on diversity

1 at sites linked to exons, and that these patterns are insensitive to recent  
2 changes in  $N_e$ .

## 3 **Results**

### 4 **Inference of selective forces operating in protein-coding genes** 5 **and CNEs**

6 As a first step to quantify and compare the selective forces acting on vari-  
7 ation in the wild rat genome, we computed nucleotide diversity ( $\pi$ ) within  
8 brown rats, nucleotide divergence ( $d$ ) from the house mouse and black rat,  
9 and Tajima's  $D$  for different classes of sites. We focused on two classes  
10 of conserved sequences: protein coding exons and CNEs. In exons, 0-fold  
11 degenerate sites are under the strongest selection, as any nucleotide change  
12 would result in an amino acid substitution. The 4-fold degenerate sites, on  
13 the other hand, are typically seen as a neutral standard for these, as mu-  
14 tations at these sites do not result in amino acid changes. CNEs are not  
15 translated to proteins, so we consider all sites within CNEs as potentially  
16 under selection for the purpose of our analysis. As a neutral standard we  
17 use CNE flanking sequence at a distance of at least 500 bp with the same  
18 total length as the element (following Halligan et al., 2013).

19 Both nucleotide diversity and divergence from the outgroups reflected  
20 this expected ranking of selection strength (fig. 1, supplementary table S1,  
21 see also figs. S1, S2, S3, S4). Diversity was lowest at 0-fold sites (0.045%),  
22 followed by CNEs (0.097%), 2-fold sites (0.112%), 4-fold sites (0.147%) and  
23 highest in CNE flanks (0.157%). Divergence from the mouse reference se-  
24 quence (mm10) followed the same rank order (3.1%, 7.1%, 10.9%, 14.2% and  
25 15.4%). In line with this, Tajima's  $D$  was similar for 0-fold sites and CNEs

1 (-0.43 and -0.41, respectively) and values for these sites were lower than  
2 at 2-fold sites, 4-fold sites and CNE flanks (-0.27, -0.23 and -0.22, respec-  
3 tively). Although all of these values are negative (implying a slight excess  
4 of low-frequency variants), they are much closer to zero than what was pre-  
5 viously found in wild mice. A recent population bottleneck would affect the  
6 genome wide diversity spectrum in a way that produces less negative, or  
7 even positive, Tajima's  $D$ . Additionally,  $\pi/d$  was higher for 0-fold sites and  
8 CNEs than for the other classes (0.015 (0-fold), 0.014 (CNEs), 0.010 (other  
9 classes)). This pattern is expected if there are slightly deleterious mutations  
10 affecting 0-fold sites and CNEs, since they are expected to contribute more  
11 to  $\pi$  than to  $d$ .

12 We then estimated distributions of fitness effects (DFE) of new mutations  
13 using the program DFE- $\alpha$  (Keightley and Eyre-Walker, 2007) and compared  
14 the results to previous estimates from wild house mice (Halligan et al., 2013)  
15 (fig. 2). DFE- $\alpha$  compares the folded site frequency spectra (SFSs) of two  
16 classes of sites, one neutrally evolving and one under selection, to assess  
17 the DFE. It uses that mutations are purged faster from the selected sites  
18 when they are more deleterious. In line with theoretical expectations for  
19 a smaller  $N_e$  in the rat, we inferred that there is a substantially larger  
20 proportion of mildly deleterious mutations ( $N_e s < 1$ ), 0.29 and 0.58 in exons  
21 and CNEs, respectively, than in the same classes in wild mice (0.17 and 0.44,  
22 respectively). Concordantly, the proportions of highly deleterious mutations  
23 ( $N_e s > 10$ ) were lower in the rat (exons: 0.65 and CNEs: 0.29) than in the  
24 mouse (0.77 and 0.37, respectively). The presence of a higher fraction of  
25 slightly deleterious mutations is consistent with the increased values of  $\pi/d$   
26 that we found for exons and CNEs.

27 We also attempted to estimate the fraction of nucleotide differences in

1 exons and CNEs driven to fixation by positive selection ( $\alpha$ ) and the rate of  
2 adaptive substitution relative to the rate of neutral substitution ( $\omega_a$ ). For  
3 this we used an extension of the McDonald-Kreitman test incorporated into  
4 DFE- $\alpha$  (McDonald and Kreitman, 1991; Eyre-Walker and Keightley, 2009).  
5 This subtracts the number of fixed nucleotide substitutions (relative to the  
6 mouse) in the selected class of sites that is expected from the fixation of  
7 neutral and deleterious mutations alone from the actual number of substi-  
8 tutions. The remainder is contributed to positive selection. We consistently  
9 obtained negative estimates for  $\alpha$  and  $\omega_a$  for both exons (table S2). As we  
10 discuss below, these negative estimates likely reflect a recent population size  
11 bottleneck.

## 12 **Reduced diversity around exons and CNEs**

13 Selection operating within CNEs and exons is also expected to affect nu-  
14 cleotide diversity in closely linked surrounding sequences as a consequence of  
15 selective sweeps (Maynard Smith and Haigh, 1974) or background selection  
16 (Charlesworth et al., 1993). We therefore investigated diversity statistics in  
17 exonic (up to 100 kb) and CNE (up to 20 kb) flanking regions, and again  
18 compared our results with those previously obtained in wild house mice  
19 (Halligan et al., 2013) (fig. 3). Although wild house mice have much higher  
20 diversity than what we observe in the rat, the relative reduction in diversity  
21 in exon flanks was remarkably similar in both species. The reduction in di-  
22 versity ( $\pi$  and  $\pi/d$ ) around CNEs, on the other hand, was less pronounced  
23 in rat. Moreover, in the bins directly adjacent to the CNEs, we found an  
24 increase of  $\pi/d$ , coinciding with a less strong reduction of  $\pi$  than in mouse  
25 at these strongly conserved sites.

26 The reduction of  $\pi$  and  $d$  in the CNE flanks appears to exist on two



length scales: a strong reduction that decays over  $\sim 500$  bp and a second less pronounced reduction that decays over tens of kb. We therefore investigated to what extent this second length scale could be the result of the proximity of CNEs to exons. We first computed the distribution of distances from CNEs to the nearest exon (figs. S5, S6). Due to the power law-like distribution of distances between neighbouring exons, bases tend to “cluster” around exons; this means that more bases are located at a particular short (e.g. 10 bp) distance from the nearest exon than at a particular large distance (e.g. 100 kb) from it (fig. 3G). Taking this into account, there remains a two-fold overrepresentation of CNEs near exons (fig. S5). Yet when we used the distribution of distances from CNEs to their nearest exon (fig. S6) to convolute ( $\approx$  blur; see methods) the exon flanks, the resulting slope “far away” from the CNEs, i.e., fitted between 5 and 20 kb away, was much shallower than the long length scale in the CNE flanks (fig. S7). This implies that the proximity to exons of many CNEs can only explain a small part of the long length scale we observed in the decay of  $\pi$  in CNE flanks.

## LD decay in rat and mouse genomes

To gain an understanding of the striking similarity of the diversity reductions in the exon flanks between rat and mouse and the less similar patterns in CNE flanks, we investigated the decay of linkage disequilibrium (LD) around focal SNPs in wild rats and mice. For this, we computed the pairwise genomic  $r^2$  (Rogers and Huff, 2009), and averaged over all SNPs at a particular distance from each focal SNP (using a bin size of 20 bp). As focal SNPs, we used either all SNPs, SNPs within exons or SNPs within CNEs (fig. 4AB).

In wild house mice, average  $r^2$  (written  $\langle r^2 \rangle$ ) decayed much faster than

1 in wild rats, and the peak value was lower, consistent with the larger  $N_e$   
2 in mice. To quantify the difference, we first fitted exponential functions to  
3 the decay of  $\langle r^2 \rangle$  with physical distance:  $f(x) = (a - c) \times \exp(-x/b) + c$ .  
4 For our purposes, characteristic length  $b$  is the biologically most important  
5 parameter, as this is the distance over which the relevant information,  $\langle r^2 \rangle -$   
6  $c$ , decays by a factor  $1/e$  (to  $\sim 37\%$  of its original value). Maximum value  $a$  is  
7 the intercept and  $c$  is the offset, which has a theoretical minimum of  $1/(n-1)$   
8 for a sample of  $n$  individuals (see supplementary text 2) and increased with  
9 decreasing  $N_e$  (Hill, 1981).

10 We found that it was impossible, however, to obtain a good fit with this  
11 kind of curve (fig. 4C). The structure of the residuals of the best fitting  
12 curves (fig. S8AB) suggested that LD ( $\langle r^2 \rangle$ ) decays first faster, then slower  
13 than exponential, which is a property of a stretched exponential  $g(x) =$   
14  $(a - c) \times \exp(-(x/b)^d) + c$ , with stretching exponent  $0 < d < 1$ . See discussion  
15 for the biological meaning of  $d$ . We obtained good fits to the data with this  
16 formula (figs. 4D, S9, table S3A). The rat:mouse ratios of  $c - 1/(n - 1)$  were  
17 all larger than 1, consistent with a larger  $N_e$  in mouse.

18 We fitted all curves again with a fixed stretch exponent of  $d = 0.5$  to allow  
19 a more direct comparison of the characteristic length parameters  $b$  (figs. 4D,  
20 S9, table S3B). This effectively exploits the fact that stretched exponentials  
21 are notoriously hard to fit to our kind of data. By these means, we found  
22 that LD decays 6-7 times faster in mouse than in rat (rat:mouse ratios of  $b$ :  
23 7.14 exons; 6.31 CNEs “noOverlap”; 5.96 CNEs “strict”; 5.76 all SNPs).

## 24 Recent bottleneck in the rat population

25 The preceding analysis provides several lines of evidence for a recent pop-  
26 ulation size bottleneck in wild rats: the much lower diversity than in wild

1 mice (figs. 1B, 3), the negative estimates of  $\alpha$  and  $\omega_a$  (table S2), the values  
2 of Tajima's  $D$  (fig. 1A) that are much larger than in mice and near zero for  
3 some functional categories of exons.

4 To further investigate the possibility of changes in population size, we  
5 used the method of Li and Durbin, PSMC, (Li and Durbin, 2011) to infer  
6 population history based on the length distribution of genome stretches that  
7 are identical by state. Based on the non-CpG prone sites, the 12 rat genomes  
8 showed a 3-fold decline in  $N_e$  up to 10,000 – 20,000 YA (fig. 5).

9 A similar trend was found using all sites (i.e., including the CpG-prone  
10 sites and adjusting mutation rates accordingly; fig. S10), using a different  
11 published mutation rate for rat of  $\mu = 4.2 \times 10^{-9}$  (Ness et al., 2012) (fig.  
12 S11B) and by varying the parameter (md) of the proximity filter (fig. S11),  
13 which is the filter that had most impact information used by PSMC, i.e.,  
14 the distribution homozygous runs (HHn from MacLeod et al., 2013, see fig.  
15 S12). For more details, see supplementary text 3.

16 From this we conclude that the rat genomes contain a strong and robust  
17 signal for a bottleneck between 10,000 and 50,000 YA. The actual bottleneck  
18 may have been sharper than the PSMC traces show – and, if it has been  
19 fairly short, also more severe – as simulations have shown that PSMC has  
20 a tendency to smoothen sharp transitions (Li and Durbin, 2011).

## 21 Discussion

22 Previous work suggests that wild brown rats have a much lower effective  
23 population size than wild house mice (Ness et al., 2012). Re-estimating  
24 the mutation rate based on our measured divergence from mouse at 4-fold  
25 degenerate sites (14.2%), assuming a divergence time of 12 MYA (Benton  
26 and Donoghue, 2007) and 2 generations per year (Halligan et al., 2013;

1 Ness et al., 2012), yields  $\mu = 2.96 \times 10^{-9}(2.94 \times 10^{-9} - 2.98 \times 10^{-9})$ .  
2 Equating  $\pi$  at 4-fold degenerate sites to  $4N_e\mu$ , we obtain an estimate of  
3  $N_e = 1.24 \times 10^5(1.20 \times 10^5 - 1.28 \times 10^5)$ , marginally smaller than the  
4  $1.3 \times 10^5$  estimate previously published. We obtained a very similar  $N_e$   
5 estimate using divergence and diversity from our other neutral class, the  
6 CNE flanking sites:  $N_e = 1.22 \times 10^5 (1.21 \times 10^5 - 1.23 \times 10^5)$  (and  $\mu =$   
7  $3.21 \times 10^{-9} (3.20 \times 10^{-9} - 3.23 \times 10^{-9})$ ).

8 From our PSMC demographic inference we obtain a minimum  $N_e$  of  
9 approximately  $4 \times 10^4$  at  $2 \times 10^4$  years ago and a 3-4 times larger ancestral  
10 size (i.e., the most ancient  $N_e$  that can be detected with this method). The  
11  $N_e$  estimate based on silent site diversity lies between these values, which is  
12 likely explained by the fact that it is affected by the whole recent population  
13 history. An effective population size of  $4 \times 10^4$  appears small for the present  
14 day, given the abundance and widespread distribution of brown rats, and the  
15 small amount of population structure that appears to be present in wild rat  
16 brown populations (Ness et al., 2012). However, it is likely that the effective  
17 size of the brown rat population has increased dramatically since the origins  
18 of human agriculture, which provided them with a large and stable source  
19 of food. None of the methods we have used, however, can reliably estimate  
20 contemporary  $N_e$ .

## 21 **Negative estimates of the rate of adaptive molecular evolution**

22 Conceptually, the rate of adaptive molecular evolution should always be a  
23 positive number. We now review the computational method to explain how  
24 a recent population bottleneck – as suggested by the unusually high values  
25 of Tajima’s  $D$  (fig. 1) and the PSMC demographic inferences (fig. 5) – could  
26 cause negative estimates of  $\alpha$ , the fraction of substitutions driven to fixation

1 by positive selection.  $\omega_a$ , the rate of adaptive substitution relative to the  
2 rate of neutral substitution, is defined such that it inherits the sign of  $\alpha$   
3 (Eyre-Walker and Keightley, 2009) OR (Halligan et al., 2013).  
4 To estimate  $\alpha$  (and  $\omega_a$ ), DFE- $\alpha$  subtracts an estimate of the fraction  
5 of (slightly) deleterious substitutions in the selected class (e.g., 0-fold sites)  
6 from the total number of substitutions in the selected class, and assumes that  
7 the remaining substitutions have been positively selected. To estimate the  
8 (slightly) deleterious fraction, the inferred DFE is used along with a single  
9 effective population size (weighted over the whole evolutionary time period  
10 under analysis), and the selected (e.g., 0-fold) and neutral (e.g., 4-fold) di-  
11 vergence from an outgroup. This implicitly assumes that the strength and  
12 effectiveness of selection (i.e., the DFE) were constant over the evolutionary  
13 time period under analysis (Eyre-Walker and Keightley, 2009). A recent  
14 population size bottleneck is expected to cause an over-representation of  
15 slightly deleterious mutations in the polymorphism data used to infer the  
16 DFE, which will therefore increase the predicted divergence in the selected  
17 site class. This implies that the DFE- $\alpha$  method, and all other similar im-  
18 plementations of the McDonald-Kreitman test, will tend to under-estimate  
19 the rate of adaptive evolution if there has been a recent population bottle-  
20 neck. At the heart of this problem lies the fact that divergence is built up  
21 continually from the moment the two lineages split (in this case 12 MYA),  
22 whereas polymorphism only reflects a limited window of recent evolution-  
23 ary history ( $4N_e$  generations). As the size of this window is dependent on  
24  $N_e$ , pre-bottleneck information is rapidly lost from polymorphism data as  
25 a consequence of a severe bottleneck. Analysing data from species with  
26 shorter divergence times may mitigate the impact of long term population  
27 size changes, albeit at the expense of power. Using *R. rattus* as an outgroup

1 could not mitigate the problem, as the split between both rat species is  $\sim 2.9$   
 2 MYA (Robins et al., 2008), i.e., long before the bottleneck we inferred from  
 3 our data. Consistently, we again obtained negative estimates of  $\alpha$  and  $\omega_a$   
 4 (table S2).

## 5 Decay of LD with physical distance

6 We inferred that there is a roughly 6-7 fold faster LD decay in wild mice  
 7 than wild rats as a function of physical distance in the genome by fitting  
 8 stretched exponential functions of the form  $(a - c) \times \exp(-(x/b)^d) + c$ . This  
 9 is consistent with a recent population size bottleneck, as confirmed using  
 10 different information such as our PSMC analysis. We found that a stretched  
 11 exponential including an offset was needed to obtain a satisfactory fit to the  
 12 data, whereas a single exponential gave a poor fit (figs. 4, S9).

13 A stretched exponential can be obtained by summing over a large number  
 14 of single exponentials with various exponents (parameter  $b$  in our functions),  
 15 (e.g., Johnston, 2006). Assuming that the recombination rate is constant  
 16 over the genome, population genetic theory predicts that LD ( $\langle r^2 \rangle$ ) decay  
 17 follows a single exponential (Charlesworth and Charlesworth, 2010), which  
 18 can be offset by the theoretical minimum for the sample size ( $1/(n - 1)$ , see  
 19 supplementary text 2) + a residual offset for the expected genome wide LD  
 20 due to finite population size (Laurie-Ahlberg and Weir, 1979; Hill, 1981).  
 21 The single exponential would also fit if fluctuations in recombination rate  
 22 average out over sufficiently short distances.

23 Previous estimates of the recombination rate in rat and mouse (Jensen-  
 24 Seaman et al., 2004), however, showed evidence of fluctuations on a scale of  
 25 much more than 10 Mb, i.e., larger than our window for calculating  $\langle r^2 \rangle$ ,  
 26 and also showed evidence for variation in whole chromosome average recom-

1    bination rate. This variation could explain the requirement for a stretched  
2    exponential. As the major fluctuations in the reported data for mouse and  
3    rat are on the same length scale, this also explains that we found smaller val-  
4    ues of the stretch exponent  $d$  for mouse than for rat (table S3A): the smaller  
5    the relevant window for LD decay, the larger the variation in the (weighted)  
6    average recombination rate over the window. The roughly 6-7 fold faster  
7    LD decay in mouse also explains why the relative differences among classes  
8    (exons, CNEs, all data) of the fitted values are larger in mouse.

9        In principle, the fitted value of the offset  $c$  minus its theoretical minimum  
10    carries information about  $N_e$  (Laurie-Ahlberg and Weir, 1979; Hill, 1981).  
11    With our window length and limitations on resolution close to the focal SNP,  
12    however, it is impossible to obtain a reliable estimate of the stretch exponent  
13     $d$  or the offset  $c$  independently. We, therefore, refrain from estimating  $N_e$   
14    in this way.

## 15    **Reductions of nucleotide diversity around protein-coding ex-** 16    **ons and CNEs**

17    A striking finding from our study is the extremely similar proportional re-  
18    ductions in mean scaled neutral nucleotide diversity around protein-coding  
19    exons in wild rats and in wild house mice (fig. 3). The depth, width and  
20    shape of the reductions in diversity are all similar. The drops in diversity  
21    are presumably caused by the hitchhiking effect of selection on variants in  
22    protein-coding exons, which reduces diversity in tightly linked flanking re-  
23    gions. The previous analysis of the pattern of diversity reduction in wild  
24    house mice suggests that there is a substantial role for selection of advanta-  
25    geous mutations, whereas a background selection model alone appears to be  
26    incapable of explaining the width of the observed mean diversity reduction

(Halligan et al., 2013). The question then arises as to whether these similar patterns around exons in the two species can be reconciled with the difference in the effectiveness of selection between the species (caused by lower  $N_e$  in rats) and the presence of substantially greater LD in rats than in mice. If diversity at linked sites is reduced by selection of newly arising advantageous mutations in exons of large selective effect(s) that go to fixation in both species (i.e., classic selective sweeps such that  $N_e s \gg 1$ ), and the rate and strength of advantageous mutations and the rate of recombination per physical distance are the same in the two species (0.555 cM/Mb in rat and 0.528 cM/Mb in mouse (Jensen-Seaman et al., 2004)), then equivalent patterns of diversity reduction are predicted, and these are not expected to depend on  $N_e$  or LD (Maynard Smith and Haigh, 1974). A similar argument can be made for the case of background selection (BGS) involving strongly deleterious mutations (Nordborg et al., 1996). Alternatively, if diversity reductions are caused by positive selection on standing variation, the pattern of diversity reduction is expected to depend on the effective population size during the phase in which a variant can rise to a high frequency by drift, and subsequently be positively selected to fixation (Przeworski et al., 2005). Specifically, a higher  $N_e$  increases the difference between the pattern of diversity change seen under a classic sweep model and a model of standing variation. The similarity of the diversity reduction patterns surrounding protein-coding exons we observe between mice and rats, species which differ substantially in recent  $N_e$ , is therefore indirect evidence in favour of the classic selective sweeps model.

Narrower and shallower scaled diversity reductions in the regions surrounding CNEs are also present, but these have somewhat different patterns in mice and rats (fig. 3). Specifically, diversity reductions are shallower in



1 rats and diversity returns to a genomic background level more slowly in rats  
2 than mice. It was previously shown in mice that the diversity reductions can  
3 be explained by a BGS model, although a role for positive selection was not  
4 excluded (Halligan et al., 2013). If diversity reductions are mainly caused  
5 by BGS, a weaker effect is expected in rats than mice if there are substantial  
6 numbers of CNE mutations which have selective effects  $< 1/N_e$  in rats and  
7  $> 1/N_e$  in mice, because these would behave as nearly neutral in rats and  
8 therefore have a smaller influence on linked neutral diversity than in mice.  
9 This is consistent with our estimates of the DFE in the two species, which  
10 suggest that there are substantially more deleterious mutations in CNEs  
11 with selective effects  $< 1/N_e$  in rats than mice (fig. 2).

## 12 Conclusion

13 We have conducted a whole genome polymorphism study to quantify the  
14 selective forces shaping recent wild brown rat evolution and compared our  
15 findings to a similar study in wild house mice. We found a larger proportion  
16 of slightly deleterious mutations in rats than in mice for both protein-coding  
17 exons and CNEs, in line with the theoretical expectation for a larger  $N_e$  in  
18 mice. The data also shows evidence for a recent population bottleneck in  
19 rats, which we dated at roughly 20,000 years ago using a PSMC analysis,  
20 followed by a likely explosion of population size starting roughly at the same  
21 time as the rise of agriculture in humans. The population size bottleneck  
22 distorted the allele frequency distribution, leading to unusually high, but still  
23 negative, Tajima's  $D$  values, and led to substantially more LD than observed  
24 in wild mice. Strikingly, however, we found a very similar pattern in the  
25 reduction of  $\pi/d$  in the tens of kbs flanking protein-coding exons, which are  
26 consistent with recurrent selective sweeps on newly arising advantageous

1 mutations.

## 2 **Methods**

### 3 **Samples**

4 We obtained genomic DNA from 22 wild *R. norvegicus* trapped in a ~500-  
5 km<sup>2</sup> area around the city of Harbin, Heilongjiang Province, China in 2011  
6 (Ness et al., 2012) from locations a minimum of 100 m apart to avoid sam-  
7 pling of closely related individuals. We selected 12 of these individuals for  
8 whole-genome sequencing. DNA from one individual black rat (*R. rattus*)  
9 that died of natural causes was obtained from Bristol Zoo's colony.

### 10 **Sequencing**

11 Genomic DNA was extracted from a small piece of kidney tissue. Standard  
12 Illumina 100bp PE libraries for the HiSeq sequencer with an insert size of  
13 approximately 450bp were prepared according to manufacturers recommen-  
14 dations. The Illumina sequencing was performed at the Wellcome Trust  
15 Sanger Institute. We obtained a modal coverage of 19x – 46x per sample  
16 for *R. norvegicus* and 33x for *R. rattus* (table S4). Reads were aligned to  
17 the rn5 reference (from Ensembl release 71) using BWA version 0.5.10-mt Li  
18 and Durbin (2009). All lanes from the same library were then merged into  
19 a single BAM file using Picard tools (<http://picard.sourceforge.net>)  
20 and PCR duplicates were marked using Picard tools 'MarkDuplicates'. Fi-  
21 nally, the library BAM files were merged into a single BAM containing all  
22 sequencing reads for that sample.

# 1 SNP calling and filtering

2 We used the Genome Analysis Toolkit (GATK) UnifiedGenotyper version  
3 2.8-1-g932cd3a for SNP calling (DePristo et al., 2011), using the following  
4 non-default arguments: output mode: emit all confident sites; genotype  
5 likelihoods model: both; stand emit conf: 10. By choosing the latter pa-  
6 rameter value, we obtained information about sites called with relatively  
7 low confidence, which were filtered subsequently, as described below.

8 Before SNP calling, we first performed indel realignment using GATK  
9 IndelRealigner with default parameters on the BAM files containing the  
10 aligned reads simultaneously on all 12 samples. There is a SNP database  
11 available for *R. norvegicus* from Ensembl, but this contains only 10% of  
12 the putative variant sites in our data (estimated from release 71, [ftp://ftp.](ftp://ftp.ensembl.org/pub/release-71/variation/vcf/rattus_norvegicus/)  
13 [ensembl.org/pub/release-71/variation/vcf/rattus\\_norvegicus/](ftp://ftp.ensembl.org/pub/release-71/variation/vcf/rattus_norvegicus/)). Re-  
14 calibrating bases using a limited SNP data set after realignment may have  
15 introduced a significant bias, so we did not do this. It has been shown,  
16 moreover, that the combination of local realignment and base recalibration  
17 is likely to result in biased SNP calls (Guo et al., 2012). We used all putative  
18 indels from a first round of SNP calling to mask all sites near putative indels  
19 (for deletions: deleted bases + 1 base on either side; for insertions: insert  
20 length + 1 base on either side of insertion point). We also performed a  
21 second round of SNP calling using the same parameters (and GATK version  
22 2.7-4-g6f46d11), but without the indel realignment step. We filtered out  
23 putative SNPs that did not appear in both sets. Both these steps were done  
24 because BWA (and any other alignment algorithm) is prone to introduce  
25 false SNPs near indels, particularly in low complexity regions (as described  
26 in the online GATK documentation). This occurs because the penalty for  
27 introducing a small number of false SNPs may be lower than the penalty

1 for introducing a gap, and is most likely to occur close to sequencing read  
2 ends.

3 We further filtered sites that had a GATK quality score  $QUAL < 23$ . This  
4 threshold was chosen post hoc, based on the distribution of scores of invari-  
5 ant sites. Our samples contained a very small fraction of invariant sites with  
6  $QUAL < 24$ , above which the density increases markedly (fig. S13). Choos-  
7 ing our threshold just below this value therefore allows filtering on quality  
8 without introducing substantial bias against invariant sites. We excluded all  
9 sites that had an inbreeding coefficient  $F < -0.8$ . GATK only computes  $F$  for  
10 a site if at least 10 samples are called at that site (online GATK documenta-  
11 tion). Using this threshold for  $F$ , all sites that have exclusively heterozygous  
12 individuals are excluded, which is a strong indication of paralogous reads  
13 mapping to the same region. Following common practice, we filtered out  
14 high and low coverage regions, since such regions are prone to SNP calling  
15 errors. We exploited the fact that we have 12 samples by applying relatively  
16 lenient bounds on a per sample basis (between 25 and 300% of the sample's  
17 modal coverage) and using much stricter bounds on the average normalized  
18 coverage (between 50 and 140%). The latter bounds were derived from the  
19 distribution of autosome wide average coverages (fig. S14). There was more  
20 than a factor of two difference between the highest and lowest modal cov-  
21 erage, so we used each individual sample's modal coverage, computed from  
22 the whole autosome, for normalizing coverage. Throughout our analyses,  
23 we only considered sites that had at least 3/12 samples called after filtering.  
24 In some analyses we applied a "**proximity filter**" that removed all variant  
25 sites less than  $md = 5$  bp from another variant site, regardless of site qual-  
26 ity. The filter does appear to cause a large number of false negatives, so we  
27 applied it only to analyses that are highly sensitive to false positives. The

1 proximity filter had a stronger impact on  $\pi$  and D than the precise selection  
2 criteria for exons/ CNEs, but had little impact on divergence statistics (figs.  
3 S1, S2, S3, S4). It never affected the rank order of different classes of sites.

4 For the *R. rattus* outgroup we used the same SNP calling pipeline, with  
5 the following exceptions: 1) we applied indel realignment to the *R. rattus*  
6 genome, aligned to the rn5 reference, in isolation. 2) we used a minimum  
7 base quality cutoff of 13 rather than the GATK default 17, based on an  
8 analysis of GC content and average base quality using Picard tools Col-  
9 lectGcBiasMetrics. 3) we used a minimum QUAL cutoff of 30, which was  
10 determined in the same way as for the *R. norvegicus* data, and is higher  
11 because of the higher modal coverage of 33x in the *R. rattus* sample. 4) we  
12 masked sites near indels using the *R. rattus* putative indels. 5) we required  
13 that sites have a normalized coverage between 40% and 200% of the *R. rat-*  
14 *tus* modal coverage. We have only one *R. rattus* sample, so filtering against  
15 likely paralogs based on Hardy-Weinberg frequencies was not possible.

16 CpG-prone sites, defined as sites preceded by a C or followed by a G,  
17 were identified based on the rn5 and mm10 reference sequences as well at  
18 the *R. norvegicus* and *R. rattus* samples. We excluded sites that were CpG-  
19 prone in any of these sequences from several of our analyses, because the  
20 hypermutability of the CG dinucleotide strongly violates the assumption of  
21 a uniform mutation rate across the genome, which is commonly made in  
22 the theory underpinning many population genetic analyses. Exclusion of  
23 CpG-prone is an effective way of removing this source of hypermutability  
24 (Gaffney and Keightley, 2008).

25 The *R. norvegicus* sample consists of four males and eight females. For  
26 consistency in filtering and statistics, we restricted all our analyses to auto-  
27 somes, and excluded unplaced contigs.

# 1 Exons

2 We used the Ensembl Rnor5.0.73 annotation file to obtain the locations of  
3 exons. We confined our analysis to exons that are part of complete tran-  
4 scripts, i.e., starting with a start codon, terminated by a stop codon and  
5 containing no premature stop codons. The annotation file contains 25,725  
6 transcripts, 20,278 of which are complete: 19,530 on the autosomes, 697  
7 on the X chromosome, 9 on the mitochondrial genome and 42 on unplaced  
8 contigs.

9 Exonic sites were analyzed only if they were consistently 0-fold, 2-fold,  
10 or 4-fold degenerate over all annotated transcripts in the rat annotation,  
11 based on computational translation of all canonical and non-canonical tran-  
12 scripts in *R. norvegicus* containing the site. Sites with inconsistent de-  
13 generacy (e.g., a site that is 4-fold degenerate in a canonical transcript, but  
14 0-fold degenerate in an overlapping non-canonical transcript) were excluded.  
15 Confidence intervals were computed using n=1,000 bootstrap replicates by  
16 sampling per transcript. For details see supplementary text 1.

# 17 Conserved noncoding elements (CNEs)

18 Noncoding sequences conserved across the mammalian phylogeny, CNEs,  
19 were defined using phastCons on a mammal phylogeny excluding rodents  
20 as described in Halligan et al. (2013). This resulted in a set of elements  
21 comprising 5% of the genome.

22 In Halligan et al. (2013), the resulting set of elements was lifted over  
23 from human hg18 coordinates to mouse mm9 using liftOver. Each liftOver  
24 step inevitably results in the loss of a subset of the elements. Simply lifting  
25 over the Halligan et al. (2013) set from mouse mm9 to rat rn5 might,  
26 therefore, result in a set biased towards higher conservation. To minimize

1 this potential bias, we used three different routes for lifting over the original  
2 hg18 coordinates to our rat rn5 reference: hg18  $\rightarrow$  hg19  $\rightarrow$  rn5; hg18  $\rightarrow$   
3 rn4  $\rightarrow$  rn5; and hg18  $\rightarrow$  mm9  $\rightarrow$  rn5. In case of conflicts, i.e., if different  
4 liftOver routes placed the same element at different positions on rn5, we only  
5 retained those that differed by no more than 25% in length from the original  
6 hg18 element. If different liftOver routes resulted in partially overlapping  
7 elements, we retained the one with length closest to the original (fig. S15).  
8 From this set of elements, we removed those segments of the elements that  
9 overlapped with annotated exons (valid and invalid). This final set of CNEs  
10 we call “noOverlap”.

11 To test how sensitive our results are to the precise definition of CNEs, we  
12 also created two slightly different sets of CNEs: “strict”: removing all ele-  
13 ments that have any overlap with annotated exons, not just the overlapping  
14 segments; and “noOverlap, <1 kb”: the noOverlap set excluding elements  
15 longer than 1kb. By analyzing the normalized CNE length distributions for  
16 different sets, we found that length distributions are very similar between  
17 the human, mouse and rat sets, with a slight inflation of very long elements  
18 in the rat (“noOverlap”) and mouse sets (fig. S15). The number of elements  
19 in these inflated tails was small, but because the median CNE length is very  
20 short, these elements, which are less strongly conserved than the average,  
21 could have a disproportionate impact on the within-CNE statistics. From  
22 the cumulative length distribution (fig. S15) it can be seen that in the human  
23 set, less than 1% of CNE bases occur in elements of over 1kb, whereas in the  
24 rat and mouse sets this is 5-6%. For this reason we used the “noOverlap,  
25 <1kb” set as default for the within-CNE statistics. For the CNE flanks there  
26 was almost no difference between both noOverlap sets, because the number  
27 of long (>1 kb) elements is only a very small fraction the total number (fig.

1 S16).

2 As a neutral reference for the CNEs, we used sequence elements 500 bp  
3 upstream and downstream of the CNE, each of half the length of the CNE  
4 (Halligan et al., 2013). From this set we masked any segments (noOverlap)  
5 or elements (strict) overlapping with exons or other CNEs (from the full  
6 noOverlap set).

7 We used a bootstrapping approach to obtain confidence intervals for the  
8 within CNE statistics. We subdivided the genome in 1Mb windows and  
9 sampled with replacement among the non-empty windows.

## 10 **Estimating the impact of exons on the diversity reduction in** 11 **CNE flanks**

12 Most CNEs occur in the vicinity of exons. We hypothesized that this ex-  
13 plains (part of) the reduction of diversity in CNE flanks, especially farther  
14 away from the CNEs. As this effect is likely stronger for CNEs close to exons  
15 than CNEs far away from them, we “blurred” the exon flanks based on the  
16 distances from CNEs and compared the slopes of the CNE flanks and the  
17 “blurred” exon flanks using a linear fit on the data 5-20 kb away from the  
18 CNEs.

19 The idea for the “blurring”, technically called convolution, is taken from  
20 image analysis and uses the same principle as simple blurring algorithms  
21 in common image manipulation programs. In those, each pixel of image is  
22 multiplied with the so called kernel, which determines how far information  
23 is smeared out over neighbouring pixels. In our case, we used the 100 bp  
24 bins with which the flanks were computed as “pixels”. Instead of a Gaussian  
25 kernel, that is typical for image blurring, we used the normalized distribution  
26 of distances of CNEs (using the “noOverlap” set) to their nearest exon (fig.



1 S6), up to a maximum of 200 kb, which covers >98.7% of all CNEs.

2 Before convolution, the exon flanks (not using the proximity filter, i.e.,  
3 md=0) were padded (extended) with 200 kb of 100 bp bins containing the  
4 average value of  $\pi$  over the regions 60-100 kb away from the exons in order  
5 to accommodate the full width of the kernel.

## 6 **Functional annotation**

7 For functional annotation of transcripts, we used the eggNog version 4.0  
8 database, downloaded from [http://eggnog.embl.de/version\\_4.0.beta/](http://eggnog.embl.de/version_4.0.beta/downloads.v4.html)  
9 [downloads.v4.html](http://eggnog.embl.de/version_4.0.beta/downloads.v4.html) on 14/01/2014 (Powell et al., 2014).

10 We combined annotations at different taxonomic levels. In cases of  
11 conflicts, we gave preference to annotations of narrower taxonomic levels  
12 (e.g. rodents over mammals), with the exception of vague annotations (“R”:  
13 “General function prediction only” or “S”: “Function unknown”). We used  
14 “X” for transcripts that did not appear in the database. Of the 25,725 tran-  
15 scripts in the annotation file, 21,242 appeared in the database, and of those  
16 16,888 had a function assigned to them (i.e., not R or S). In a subsequent  
17 analysis we found that the group of X labeled transcripts was a consistent  
18 outlier compared to all other annotations on a variety of metrics. We there-  
19 fore repeated the within-exon analysis excluding these transcripts to assure  
20 that our results do not hinge on this specific subset.

## 21 **Genome-wide LD scans**

22 For each variant site (“focal SNP”) we computed the pairwise  $r^2$  statistic  
23 directly from the genotypic data (Rogers and Huff, 2009) using up to  $n_{max}$   
24 = 1,500 neighbouring variant sites and sites up to 40 kb away from the focal  
25 SNP. We collected averages in bins of 20 bp. The value of  $n_{max}$  was chosen

1 such that it would not lead to the exclusion of sites from the analysis. This  
2 value allows for an average diversity of 3.75% within the 40kb, >20 times  
3 the average value we found at 4-fold synonymous sites and in 500 bp outside  
4 CNEs (fig. 1), before excluding sites. To check this, we verified that the  
5 number of sites in the most distant bins did not increase by further increasing  
6  $n_{max}$  in a preliminary analysis using the entire chromosome 19. Note that  
7 from the highest diversity estimates reported here, the expected number  
8 of SNPs within 40kb would be more than an order of magnitude smaller  
9 than our  $n_{max}$ , leaving a wide margin for variation in SNP density. We  
10 only considered biallelic sites for which all samples were called and passed  
11 the default filters. Moreover, we applied the proximity filter with md=5 for  
12 this analysis, because without it we observed small a depression in the  $\langle r^2 \rangle$   
13 curves within 200 bp of the focal SNP, whereas theory predicts a monotonic  
14 decline (fig. S8C). This suggests that a fraction of the spurious SNPs that  
15 are removed by the proximity filter are of a different kind than the genuine  
16 SNPs, which results in a population of sites with much faster decay of  $\langle r^2 \rangle$   
17 than the others. Sites near indels and CpG-prone sites were not considered  
18 in this analysis.

## 19 **Genome wide LD scans in *M. m. castaneus***

20 Genotypes were obtained from the VCF files generated by (Halligan et al.,  
21 2013). To treat the data as similarly as possible to the rat data, we calcu-  
22 lated modal coverage per sample from per sample coverage histograms for  
23 the autosome and applied the same bounds as on the *R. norvegicus* data:  
24 average normalized coverage between 50 and 140% and per sample between  
25 25 and 300%. We also determined quality histograms for variant and invari-  
26 ant sites, from which we obtained a minimum quality score of 15. Following

1 the original analysis, we disregarded sites with a  $\chi^2$  score for HW equilib-  
2 rium  $\geq 0.0002$  (against paralogs) and near indels. We removed CpG prone  
3 sites based on CpG prone status in *M. m. castaneus*, *M. m. famulus* and  
4 *R. norvegicus* (rn4). As with the rat data, all samples had to pass all fil-  
5 ters. The mouse sample has a much higher sequence diversity than the rat  
6 sample, so to cut computational cost, and because a preliminary analysis  
7 showed a much faster decay of  $\langle r^2 \rangle$  than in rat, we only considered sites  
8 up to 20 kb away from the focal site. We used  $n_{max} = 7,500$ , equivalent  
9 to 15,000 on 40kb or 10x the  $n_{max}$  used for rat, whereas the difference in  
10 diversity in regions  $>80$  kb from exons is less than five fold. The proximity  
11 filter had virtually no impact on the mouse data (fig. S8C).

## 12 Inference of population history

13 We used the inference method and software Pairwise Sequentially Markovian  
14 Coalescent, PSMC from (Li and Durbin, 2011). For prediction of population  
15 size at the most recent time scales, this method is known to be sensitive to  
16 false positive heterozygote sites (MacLeod et al., 2013). We therefore re-  
17 quired that at least 10/12 individuals were called, so that inbreeding coeffi-  
18 cient estimates are available in the GATK output. We applied the proximity  
19 filter with md=5 (and md=10 for further sensitivity analysis) and at the in-  
20 dividual sample level we only considered calls with a genotype quality  $\geq 20$ .  
21 Following (Li and Durbin, 2011), we binned the genome into 100 bp windows  
22 and created a fasta-like sequence consisting of the letters “K” (at least one  
23 heterozygous site in the bin), “T” (no heterozygous sites) and “N” (less than  
24 10% of sites in the bin called and remaining after filtering). These sequences  
25 were then directly input to PSMC using the following arguments: -N80 -  
26 r0.63 (for not CpG-prone only) or 1.33 (all sites) -t15 -p “2\*4+18\*2+4+6”.

1 These parameters differ from the defaults recommended for the analysis of  
2 human data in the PSMC documentation <https://github.com/lh3/psmc>  
3 in three ways. First, we used a much larger number of iterations, after find-  
4 ing that the default ( $N=25$ ) was insufficient for convergence (convergence  
5 of all inferences checked in fig. S17). Second, we reduced the number of  
6 free parameters to prevent overfitting (default: -p “4+25\*2+4+6”). Third,  
7 we initiated  $r$ , the ratio  $\theta/\rho$ , to per base pair estimates of  $\mu/c$  (default:  
8  $r=5$ ). Parameter  $t$  (initial value for history length in units of number of  
9 generations/ $2N_0$ ) was taken from the online documentation, claimed to give  
10 good results on human data. The program failed to produce meaningful  
11 results using a higher starting value of  $t=20$ .

12 To scale the inferred demographic histories to real time, we assumed a  
13 recombination rate of 0.6 cM/Mb (Jensen-Seaman et al., 2004) and a com-  
14 bination of a generation time of 0.5 years (Ness et al., 2012) and a mutation  
15 rate of  $2.96 \times 10^{-9}$  per base pair (calculated from our data of divergence  
16 from mouse on 4-fold synonymous sites) on the data excluding CpG-prone  
17 sites and  $5 \times 10^{-9}$  or  $8 \times 10^{-9}$  on the full sequence data to reflect the higher  
18 mutation rate of CpG-prone sites. The latter mutation rate corresponds,  
19 for example, to a 2.7 or 5.3 times higher mutation rate, respectively, and  
20 CpG-prone sites taking up 40% of the genome and was chosen because it  
21 produced either same length genealogies ( $5 \times 10^{-9}$ ) or had the main local  
22 minimum coinciding in time ( $8 \times 10^{-9}$ ). As the model assumes that the mu-  
23 tation rate is constant over time and throughout the genome, we considered  
24 inferences based on all sequence as less reliable than those based on the  
25 non-CpG prone sites, but did produce them as a qualitative control. For  
26 details on testing the robustness of the inference, see supplementary text 3.

## 1 Data access

2 FastQ and BAM files containing all reads aligned to the rn5 reference genome  
3 are deposited in the European Nucleotide Archive as study ERP001276  
4 <http://www.ebi.ac.uk/ena/data/view/ERP001276&display=html>.

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11 Zoo) for providing us with a *R. rattus* sample.

## 12 Disclosure declaration

13 The authors declare no conflicts of interest.

# 1 Figure legends

## 2 List of Figures

3	1	Tajima's $D$ (A), diversity (B) and divergence from mouse	
4		(mm10; C) and <i>R. Rattus</i> (D) for wild <i>R. norvegicus</i> data.	
5		Error bars show 95% confidence intervals based on 1000 boot-	
6		strapping replicates. See supplementary material for impact	
7		of proximity filter, exon/CNE selection rules (figs. S1, S2, S3	
8		and S4) and restricting analysis to sites with corresponding	
9		<i>R. rattus</i> bases (table S1). . . . .	34
10	2	DFE of deleterious mutations for rat exons (red), mouse exons	
11		(blue), rat CNEs (magenta) and mouse CNEs (cyan). Error	
12		bars indicate 95% confidence intervals from 1000 bootstrap-	
13		ping replicates. Mouse data after Halligan et al. (2013). . . .	35



1	5	Inferred population history from distribution of IBS (identical	
2		by state) tract length distributions Li and Durbin (2011).	
3		Curves represent the inferred population history of individual	
4		rat samples. Estimates are based on non-CpG prone sites only	
5		and using a proximity filter of $md = 5$ bp. see figs. S10 and	
6		S11 for effects of these choices. . . . .	38



# <sup>1</sup> Figures

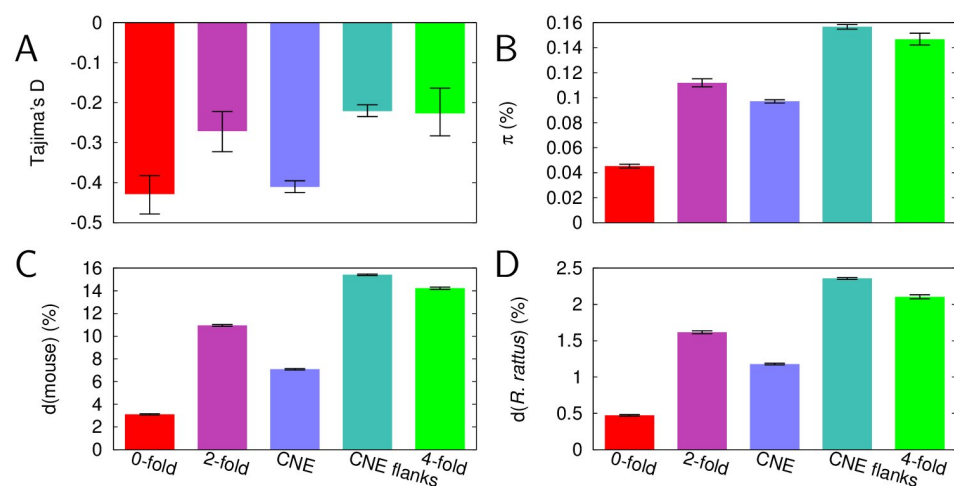


Figure 1

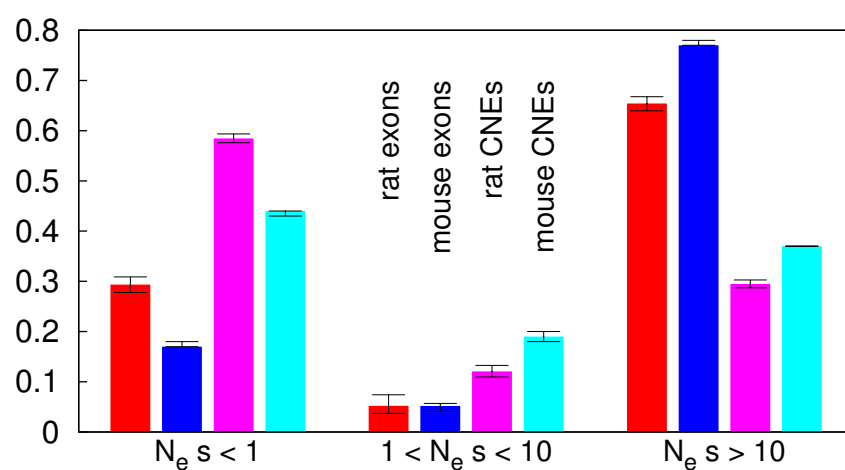


Figure 2

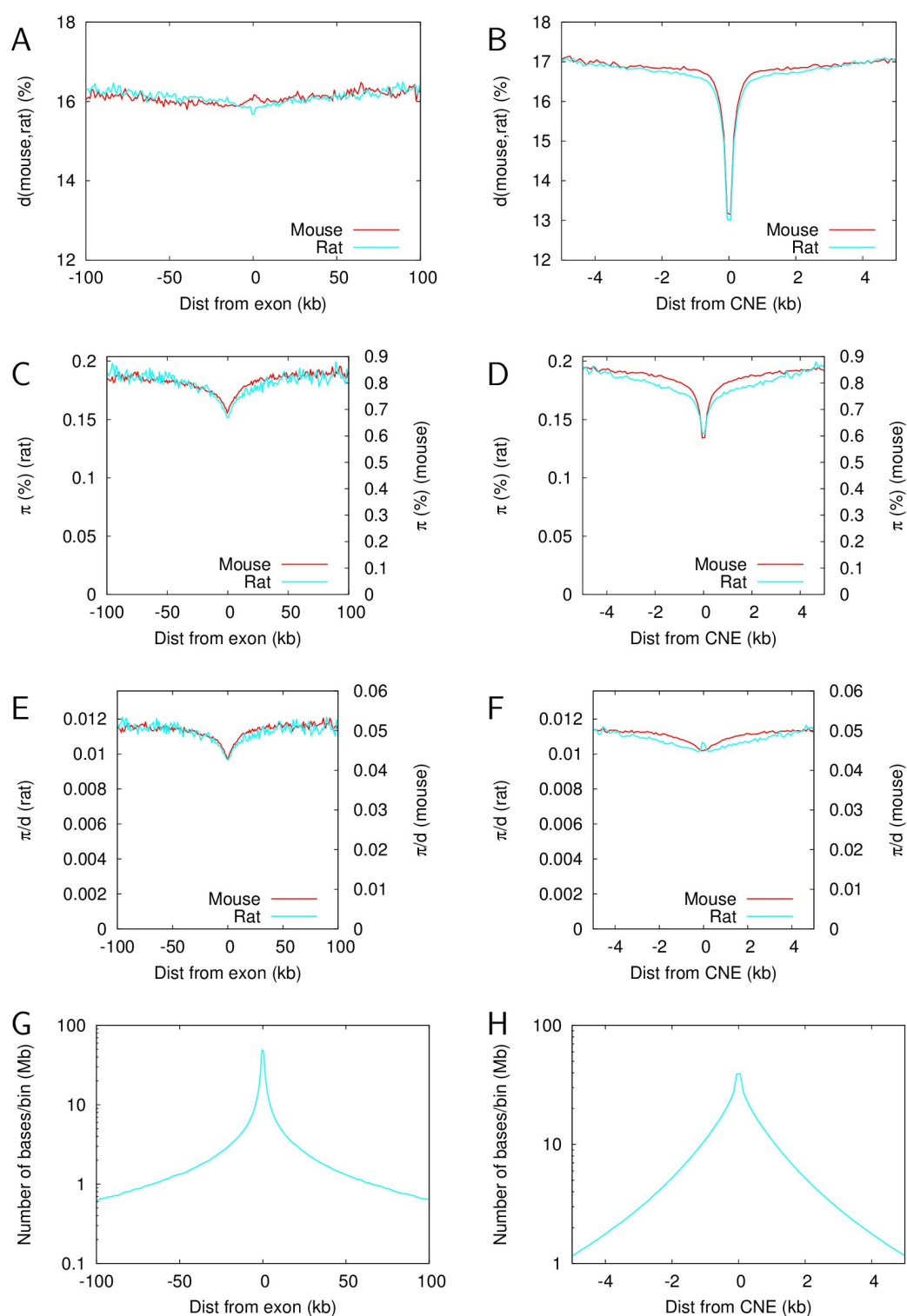


Figure 3

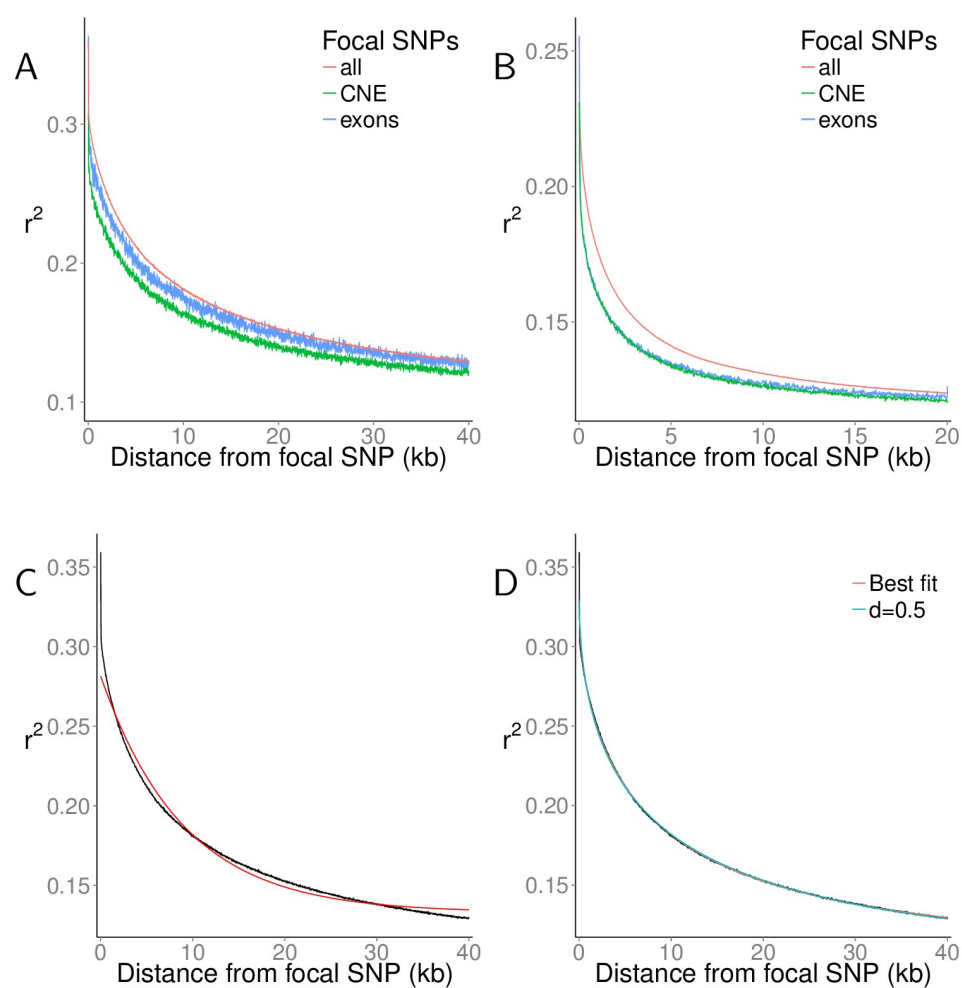


Figure 4

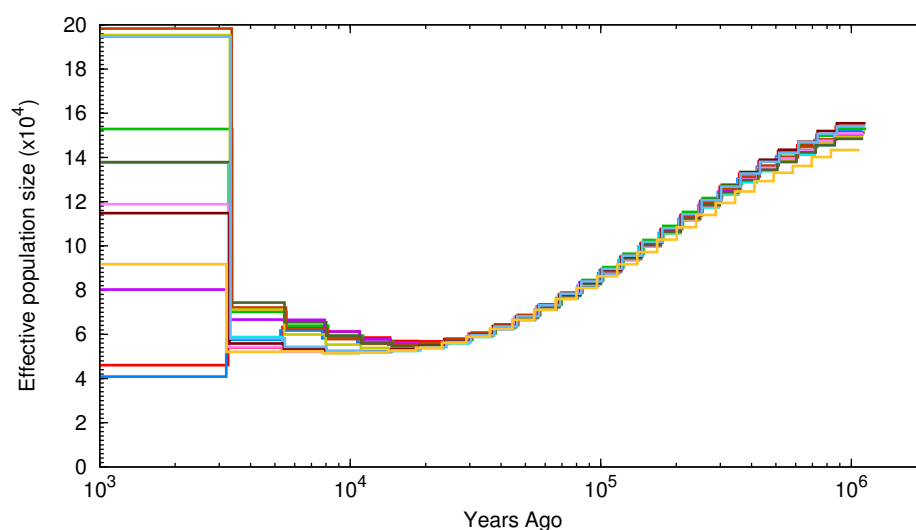


Figure 5

# 1 Tables

# 2 References

- 3 Baines JF and Harr B, 2007. Reduced x-linked diversity in derived popula-  
4 tions of house mice. *Genetics*, **175**(4):1911–1921.
- 5 Begun DJ, Holloway AK, Stevens K, Hillier LW, Poh Y-P, Hahn MW, Nista  
6 PM, Jones CD, Kern AD, Dewey CN, *et al*, 2007. Population genomics:  
7 whole-genome analysis of polymorphism and divergence in drosophila sim-  
8 ulans. *PLoS Biol*, **5**(11):e310.
- 9 Bejerano G, Pheasant M, Makunin I, Stephen S, Kent WJ, Mattick JS, and  
10 Haussler D, 2004. Ultraconserved elements in the human genome. *Science*,  
11 **304**(5675):1321–1325.
- 12 Benton MJ and Donoghue PCJ, 2007. Paleontological evidence to date the  
13 tree of life. *Mol Biol Evol*, **24**(1):26–53.
- 14 Cai JJ, Macpherson JM, Sella G, and Petrov DA, 2009. Pervasive hitchhik-  
15 ing at coding and regulatory sites in humans. *PLoS Genet*, **5**(1):e1000336.
- 16 Charlesworth B and Charlesworth D, 2010. *Elements of evolutionary genet-*  
17 *ics*. Roberts and Company Publishers.
- 18 Charlesworth B, Morgan MT, and Charlesworth D, 1993. The effect of dele-  
19 terious mutations on neutral molecular variation. *Genetics*, **134**(4):1289–  
20 1303.
- 21 DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C,  
22 Philippakis AA, del Angel G, Rivas MA, Hanna M, *et al*, 2011. A frame-  
23 work for variation discovery and genotyping using next-generation dna  
24 sequencing data. *Nat Genet*, **43**(5):491–498.

1 Eyre-Walker A and Keightley PD, 2009. Estimating the rate of adaptive  
2 molecular evolution in the presence of slightly deleterious mutations and  
3 population size change. *Mol Biol Evol*, **26**(9):2097–2108.

4 Gaffney DJ and Keightley PD, 2008. Effect of the assignment of ancestral  
5 cpg state on the estimation of nucleotide substitution rates in mammals.  
6 *BMC Evol Biol*, **8**:265.

7 Guo Y, Li J, Li C-I, Long J, Samuels DC, and Shyr Y, 2012. The effect  
8 of strand bias in illumina short-read sequencing data. *BMC Genomics*,  
9 **13**:666.

10 Halligan DL, Kousathanas A, Ness RW, Harr B, Ery L, Keane TM, Adams  
11 DJ, and Keightley PD, 2013. Contributions of protein-coding and reg-  
12 ulatory change to adaptive molecular evolution in murid rodents. *PLoS*  
13 *Genet*, **9**(12):e1003995.

14 Hernandez RD, Kelley JL, Elyashiv E, Melton SC, Auton A, McVean G,  
15 Project G, Sella G, and Przeworski M, 2011. Classic selective sweeps  
16 were rare in recent human evolution. *Science*, **331**(6019):920–924.

17 Hill WG, 1981. Estimation of effective population size from data on linkage  
18 disequilibrium. *Genetical Research*, **38**(03):209–216.

19 Jensen-Seaman MI, Furey TS, Payseur BA, Lu Y, Roskin KM, Chen C-  
20 F, Thomas MA, Haussler D, and Jacob HJ, 2004. Comparative recom-  
21 bination rates in the rat, mouse, and human genomes. *Genome Res*,  
22 **14**(4):528–538.

23 Johnston D, 2006. Stretched exponential relaxation arising from a continu-  
24 ous sum of exponential decays. *Physical Review B*, **74**(18):184430.



1 Keightley PD and Eyre-Walker A, 2007. Joint inference of the distribution of  
2 fitness effects of deleterious mutations and population demography based  
3 on nucleotide polymorphism frequencies. *Genetics*, **177**(4):2251–2261.

4 Laurie-Ahlberg C and Weir B, 1979. Allozymic variation and linkage dis-  
5 equilibrium in some laboratory populations of drosophila melanogaster.  
6 *Genetics*, **92**(4):1295–1314.

7 Li H and Durbin R, 2009. Fast and accurate short read alignment with  
8 burrows-wheeler transform. *Bioinformatics*, **25**(14):1754–1760.

9 Li H and Durbin R, 2011. Inference of human population history from  
10 individual whole-genome sequences. *Nature*, **475**(7357):493–496.

11 MacLeod IM, Larkin DM, Lewin HA, Hayes BJ, and Goddard ME, 2013. In-  
12 ferring demography from runs of homozygosity in whole-genome sequence,  
13 with correction for sequence errors. *Mol Biol Evol*, **30**(9):2209–2223.

14 Maynard Smith J and Haigh J, 1974. The hitch-hiking effect of a favourable  
15 gene. *Genetical research*, **23**(01):23–35.

16 McDonald JH and Kreitman M, 1991. Adaptive protein evolution at the  
17 adh locus in drosophila. *Nature*, **351**(6328):652–654.

18 Mouse Genome Sequencing Consortium, 2002. Initial sequencing and com-  
19 parative analysis of the mouse genome. *Nature*, **420**(6915):520–562.

20 Ness RW, Zhang Y-H, Cong L, Wang Y, Zhang J-X, and Keightley PD, 2012.  
21 Nuclear gene variation in wild brown rats. *G3 (Bethesda)*, **2**(12):1661–  
22 1664.

23 Nordborg M, Charlesworth B, and Charlesworth D, 1996. The effect of  
24 recombination on background selection. *Genet Res*, **67**(2):159–174.

- 1 Phifer-Rixey M, Bonhomme F, Boursot P, Churchill GA, Pilek J, Tucker PK,  
2 and Nachman MW, 2012. Adaptive evolution and effective population size  
3 in wild house mice. *Mol Biol Evol*, **29**(10):2949–2955.
- 4 Powell S, Forslund K, Szklarczyk D, Trachana K, Roth A, Huerta-Cepas  
5 J, Gabaldn T, Rattei T, Creevey C, Kuhn M, *et al*, 2014. egglog v4.0:  
6 nested orthology inference across 3686 organisms. *Nucleic Acids Research*,  
7 **42**(D1):D231–D239.
- 8 Przeworski M, Coop G, and Wall JD, 2005. The signature of positive selec-  
9 tion on standing genetic variation. *Evolution*, **59**(11):2312–2323.
- 10 Robins JH, McLenachan PA, Phillips MJ, Craig L, Ross HA, and Matisoo-  
11 Smith E, 2008. Dating of divergences within the rattus genus phylogeny  
12 using whole mitochondrial genomes. *Molecular Phylogenetics and Evolu-  
13 tion*, **49**(2):460 – 466.
- 14 Rogers AR and Huff C, 2009. Linkage disequilibrium between loci with  
15 unknown phase. *Genetics*, **182**(3):839–844.
- 16 Salcedo T, Geraldles A, and Nachman MW, 2007. Nucleotide variation in  
17 wild and inbred mice. *Genetics*, **177**(4):2277–2291.
- 18 Sattath S, Elyashiv E, Kolodny O, Rinott Y, and Sella G, 2011. Pervasive  
19 adaptive protein evolution apparent in diversity patterns around amino  
20 acid substitutions in drosophila simulans. *PLoS Genet*, **7**(2):e1001302.
- 21 Tange O, 2011. Gnu parallel - the command-line power tool. *;login: The  
22 USENIX Magazine*, **36**(1):42–47.