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Report

Avoidable false PSMC population size peaks occur across numerous studies

Highlights

- We detected and solved a common artifact in PSMC and related methods
- Default PSMC parameters frequently cause false peaks in recent population histories
- New methods perform better but do not always avoid this artifact
- Users should test multiple parameter settings that split the first time window

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In brief

Hilgers et al. detect and solve a common artifact in PSMC and related methods for historical population size inference. Analyses of real and simulated genomes show that ill-set default parameters falsely infer recent population peaks followed by population collapses, which can be avoided by adjusting parameter settings.

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Report

Avoidable false PSMC population size peaks occur across numerous studies

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SUMMARY

Inferring historical population sizes is key to identifying drivers of ecological and evolutionary change and crucial to predicting the future of species on our rapidly changing planet. The pairwise sequentially Markovian coalescent (PSMC) method provided a revolutionary framework to reconstruct species' demographic histories over millions of years based on the genome sequence of a single individual. Here, we detected and solved a common artifact in PSMC and related methods: recent population peaks followed by population collapses. Combining real and simulated genomes, we show that these peaks do not represent true population dynamics. Instead, ill-set default parameters cause false peaks in our own and published data, which can be avoided by adjusting parameter settings. Furthermore, we show that certain changes in population structure can cause similar patterns. Newer methods, like Beta-PSMC, perform better but do not always avoid this artifact. Our results suggest testing multiple parameters that split the first time window before interpreting recent population peaks followed by collapses and call for the development of robust methods.

RESULTS AND DISCUSSION

The pairwise sequentially Markovian coalescent (PSMC) method provides information on past population expansions, contractions, and bottlenecks. To infer changes in effective population size (Ne) over time, PSMC estimates coalescent rates, which are translated into a demographic trajectory assuming neutral evolution under random mating in a single population. PSMC analysis has become a routine analysis for newly sequenced genomes and has been used to elucidate fundamental questions regarding the evolutionary history of many organisms, including humans,[1] historical drivers of contemporary extinction risk,[2] and the consequences of climate change.[3,4] In our investigations of turtle population histories with PSMC, we detected a curious pattern of dramatic peaks followed by even more extreme population collapses (Figures 1A–1F). This pattern occurred across distantly related turtle species around the globe and its existence was consistently supported based on bootstrap replicates. Surprisingly, similar patterns are common in the literature and several studies linked them to climatic or ecological change. For example, an extreme Ne increase followed by a more than 100-fold population collapse in horses around the last glacial maximum was linked to expanding and contracting grasslands.[5] Similar peaks were further interpreted as indicating the colonization of Southern Africa by Cape Buffalo,[6] or a transition from the initially positive effects of human agriculture to increased hunting pressure on Pink-Footed Goose.[7] In total, our non-exhaustive search revealed >70 studies with >200 individual PSMC inferences exhibiting extreme peaks followed by population collapses (Table S1). This very pronounced and consistent pattern found in species from across the tree of life raises questions about its biological plausibility and points to a possible presence of analytical artifacts. Such artifacts could cause far-reaching wrong conclusions regarding evolutionary processes, effects of climate change and species conservation.

To investigate this, we first asked whether the observed peaks can represent true population size changes. To this end, we simulated diploid genomes (30 replicates) from a population

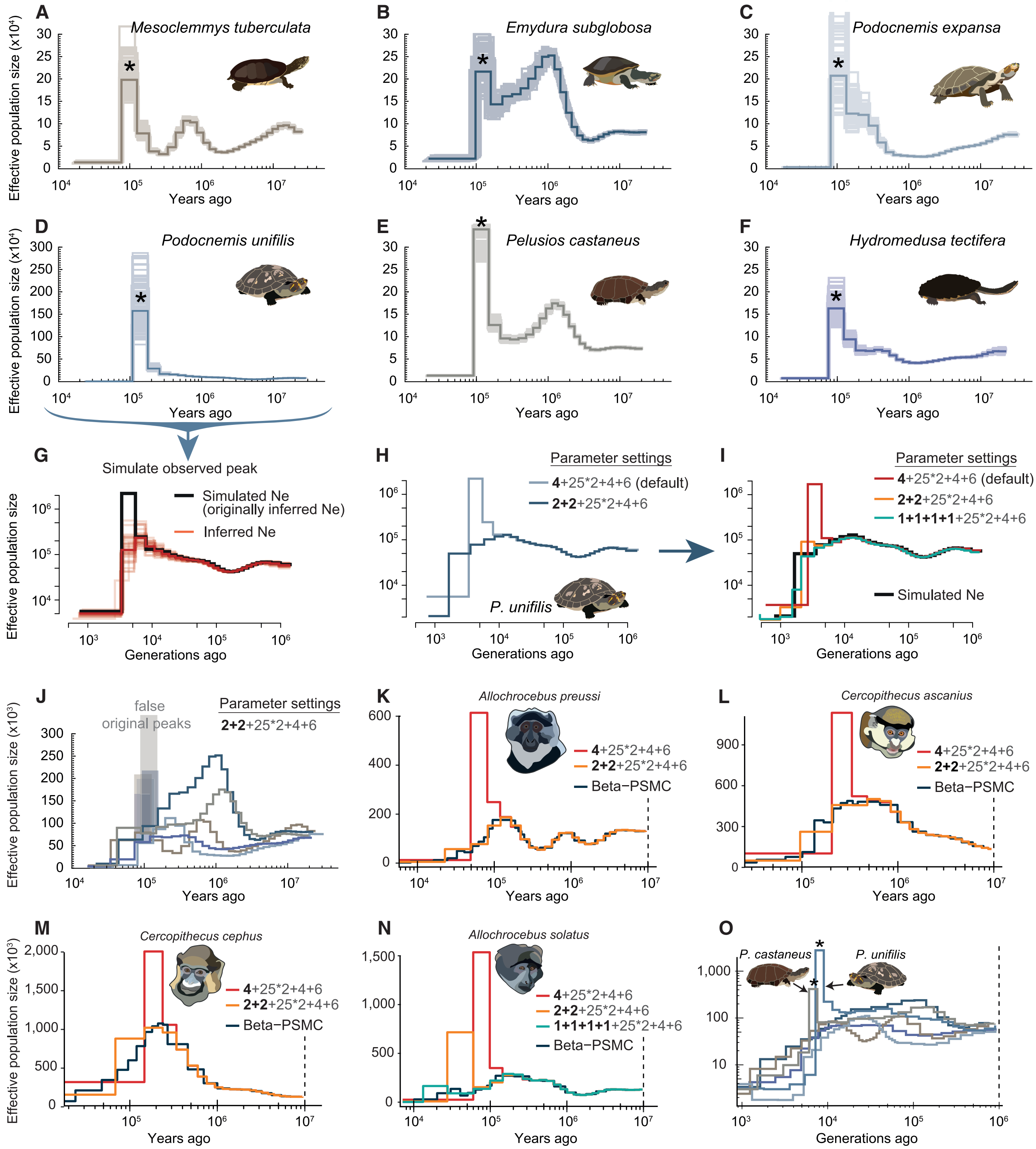

Figure 1. Erroneous peaks with PSMC default settings

(A–F) Effective population size (Ne) inferences for turtles with extreme Ne peaks before population collapses (asterisks) ~100,000 years ago. Ne is shown on the y axis and time in years on the x axis. Faint lines indicate uncertainty of inferred Ne based on 100 bootstrap replicates.

(G) Mean Ne inference with PSMC (red) based on 30 simulated genomes (pale red) from a population following the Ne trajectory of *P. unifilis* in (D) (black).

(H) Splitting the first time window (dark blue) removes the extreme Ne peak inferred in *P. unifilis*.

(I) Mean inferred Ne using different parameter settings on 30 simulated genomes with the population history (black) inferred for *P. unifilis* using the split parameter setting (see H).

(J) Splitting the first time window removes erroneous peaks inferred with PSMC default parameters (faint boxes) in all of our turtle species.

(legend continued on next page)

that followed the exact inferred population history of the turtle species with the most pronounced peak in our data: *Podocnemis unifilis* (*P. unifilis*) (Figure 1D). Notably, applying PSMC to these genomes, we never detected the extreme Ne peak that we simulated (Figure 1G). This result makes it unlikely that the observed peaks reflect true Ne peaks.

Second, we considered population fragmentation before a population decline as a possible cause. In such a scenario, a single panmictic population would split into subpopulations with limited migration and thus reduced gene flow (Figure S1). Because alleles cannot coalesce as long as they are limited to different subpopulations, the chance of coalescence is reduced during time periods with increased population fragmentation. Because PSMC assumes random mating, reduced coalescence rates caused by population structure will be falsely interpreted as a signature of larger population sizes.[1,4,8]

To test whether an increase in population structure, not size, could explain the extreme peaks, we used a similar simulation as above but kept the population size constant before it declines. At the time of the inferred peak in *P. unifilis*, we fragmented the previously panmictic population into three subpopulations, with effective migration rates (M) ranging from low (M = 0.01) to high (M = 10) (Figure S1). Our simulations show that population fragmentation caused a peak that became more prominent with increasing M, approximating the observed original peak at M = 5. This shows that certain changes in population structure followed by a population collapse can cause patterns resembling the observed peaks. However, it seems unlikely that these processes would always occur in the first and second PSMC time window.

Third, we considered the possibility of a technical artifact related to PSMC parameter settings. Although several studies have used alternate settings, parameters optimized for the human[1] are commonly used as default. Most importantly, this default setting fixes the first four atomic time intervals into the first time window. This means PSMC infers a single Ne for a large first time window and cannot model population size changes during this time. For cases with population declines within this window, we reasoned that the model may overcompensate for fixed low Ne by producing exaggerated Ne estimates in the previous time window.

To test this, we applied PSMC to the real *P. unifilis* data but split the first time window into two windows ("-p **2** + **2** + 25 × 2 + 4 + 6" instead of the default "-p **4** + 25 × 2 + 4 + 6"). In line with our hypothesis, this parameter change completely removes the Ne peak (Figure 1J). To make sure that this behavior is independent of specific genomic features of *P. unifilis*, we simulated 30 genomes that underwent a rapid population decline without a prior radical population size increase, as inferred for *P. unifilis* with split parameter settings (see Figure 1H). Using these genomes, we ran PSMC and found that the artifactual peak was only inferred with the default parameter setting (Figure 1I). This supports the idea that a technical artifact causes erroneous Ne peaks in the second time interval because a population decline within the fixed first time window cannot be adequately captured by the model. Consistently, splitting the first time window also removed the erroneous peaks in all other turtle species (Figure 1J). Aside from this recent time period, a wide range of settings on both simulated data and real data from humans, other primates, and turtles revealed highly congruent results (Figures S1D–S1G).

To confirm that similar peaks in published datasets are also artifacts caused by false parameter settings, we reran PSMC for four primate species exhibiting similar Ne peaks.[9] As expected, splitting the first time window also removes the peak originally observed for these species (Figures 1K–1N). In one species, the population decline reached into the first two atomic time intervals so that the peak only disappeared when the first time window was split into four (-p **1 + 1 + 1 + 1** + 25 × 2 + 4 + 6) (Figure 1N).

Since the publication of the original PSMC method, related methods have been developed. However, among the most recent developments, MSMC2 sometimes produces similar extreme peaks.[9] To test whether other methods also generate erroneous peaks, we ran the recently published Beta-PSMC, which should perform better in recent time intervals.[10] Beta-PSMC outputs for turtle genomes and published primate genomes rarely showed erroneous peaks, however, with a few exceptions (Figures 1K–1O). For the turtles *P. unifilis* and *Pelusios castaneus*, we also observed erroneous peaks with Beta-PSMC (Figure 1O), indicating that this approach does not fully solve the issue.

In conclusion, our results indicate avoidable false population peak inferences across numerous studies, caution against overinterpreting sharp population increases followed by a drastic decline in recent times, and provide a guideline to avoid these artifacts. First, with PSMC, we recommend testing multiple parameter settings that split the first window. Reliable inferences should not be sensitive to different parameter settings. Thus, if a peak is only observed with the default parameter setting, it is likely an artifact. Although bootstrapping is used to assess robustness across genomic regions, robustness to different parameters should also be assessed and reported. Second, population structure can be a potential driver of inferred Ne peaks. Third, newer methods such as Beta-PSMC avoid many but not all of these artifacts. In addition to the specific tools we investigated, our findings are likely to also affect other tools that use PSMC results as input. Our results motivate the development of approaches that solve the problem entirely. For example, it is conceivable to dynamically and automatically split time windows into smaller intervals, as long as enough data per window are available for a robust inference of Ne.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Leon Hilgers (leon.hilgers@senckenberg.de).

(K–N) Effective Ne inferences for four primate species using several PSMC parameter settings and Beta-PSMC. PSMC default settings recapitulated published peaks (red). Both Beta-PSMC (dark blue) and splitting the first window (yellow and turquoise) removed the erroneous Ne peaks.
(O) Beta-PSMC results for turtle species using the color code from (A)–(F). Asterisks mark erroneous peaks in two turtles. To aid readability, we excluded very large Ne estimates by Beta-PSMC for the oldest time windows (dashed lines).
See also Figure S1.

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data needed to rerun analyses, including the simulated genome data, are openly available at figshare.com (DOI: https://doi.org/10.6084/m9.figshare.26975500).
- The original code for running genome simulations is openly available at figshare.com (DOI: https://doi.org/10.6084/m9.figshare.26975500).
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization, L.H. and M.H.; methodology, L.H., S.L., and A.J.; investigation, L.H.; formal analysis, L.H., S.L., and A.J.; writing – original draft, L.H. and M.H.; writing – review & editing, L.H., S.L., A.J., T.B., T.C, R.S., K.G., and M.H.; visualization, L.H. and A.J.; funding acquisition, M.H.; resources, M.H.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Mesoclemmys tuberculata genome assembly	The McDonnell Genome Institute, Washington University School of Medicine	GenBank: GCA_007922155.1
Podocnemis expansa genome assembly	The McDonnell Genome Institute, Washington University School of Medicine	Genbank: GCA_007922195.1
Emydura subglobosa genome assembly	The McDonnell Genome Institute, Washington University School of Medicine	GenBank: GCA_007922225.1
Pelusios castaneus genome assembly	The McDonnell Genome Institute, Washington University School of Medicine	GenBank: GCA_007922175.1
Human resequencing data	1000 Genomes Project	HGDP00551, HGDP00931
Macaca mulatta genome assembly	The Genome Institute at Washington University School of Medicine	RefSeq: GCF_003339765.1 (Mmul_10)
Turtle population size inferences	This study	https://figshare.com/articles/dataset/PSMCFA_files_and_code_zip/26975500?file=49094971 https://doi.org/10.6084/m9.figshare.26975500
Primate population size inferences	This study	https://figshare.com/articles/dataset/PSMCFA_files_and_code_zip/26975500?file=49094971 https://doi.org/10.6084/m9.figshare.26975500
Human population size inferences	This study	https://figshare.com/articles/dataset/PSMCFA_files_and_code_zip/26975500?file=49094971 https://doi.org/10.6084/m9.figshare.26975500
Simulated genome sizes	This study	https://figshare.com/articles/dataset/PSMCFA_files_and_code_zip/26975500?file=49094971 https://doi.org/10.6084/m9.figshare.26975500
Code for genome simulations	This study	https://figshare.com/articles/dataset/PSMCFA_files_and_code_zip/26975500?file=49094971 https://doi.org/10.6084/m9.figshare.26975500
Software and algorithms		
Longranger	10X Genomics	https://github.com/10XGenomics/longranger
samtools	Danecek et al.[11]	https://samtools.github.io
PSMC	Li & Durbin[1]	https://github.com/lh3/psmc
GATK	Van der Auwera & O'Connor[12]	https://github.com/broadinstitute/gatk
SNPable	Li et al.	https://lh3lh3.users.sourceforge.net/snpable.shtml
Beta-PSMC	Liu et al.[10]	https://github.com/ChenHuaLab/Beta-PSMC
Picard	Broad Institute	https://github.com/broadinstitute/picard
Generating samples under a Wright-Fisher neutral model of genetic variation	Hudson[13]	https://snoweye.github.io/phyclust/document/html/ms.html

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Data used in this study includes published human data, published genomes of *A. solatus*, *A. preussi*, *C. cephus*, *C. ascanius*, *M. tuberculata*, *P. expansa*, *E. subglobosa* and *P. castaneus*, as well as newly generated high-quality chromosome-level reference genomes of *H. tectifera* (female from Turtle Island, Graz, Austria) and *P. unifilis* (male from Leipzig Zoo, Germany). *P. unifilis* and *H. tectifera* have been in captivity in Europe since before 2014.

METHOD DETAILS

Read mapping and variant calling

For turtles, 10X reads were mapped to published genomes of *M. tuberculata* (GenBank: GCA_007922155.1), *P. expansa* (GenBank: GCA_007922195.1), *E. subglobosa* (GenBank: GCA_007922225.1) and *P. castaneus* (GCA_007922175.1), as well as newly generated high-quality chromosome-level reference genomes of *H. tectifera* and *P. unifilis* (BUSCO completeness >99.5%, Contig N50 > 30MB) using Longranger (v2.2.2). Specifically, the longranger align command was used to group reads based on the 10X barcodes, trim these barcodes from the input sequences, perform error-correction, sort and align the reads to the genomes. Resulting mappings were quality adjusted (mpileup -C50), filtered to remove sites with less than one third or more than twice the average genome wide coverage with bcftools (v1.10.2).[11] Mean coverages raged from 48X to 85X (*E. subglobosa*: 48X, *M. tuberculata*: 54X, *P. castaneus*: 52X, *P. unifilis*: 85X, *P. expansa*: 48X, *H. tectifera*: 76X). Finally, variant calls (including invariant sites) were converted to fasta sequences and the PSMC-specific input format using fq2psmcfa (-q20) (https://github.com/lh3/psmc).

The primate genomes (Illumina paired end 150 bp, ~30X coverage) were processed following the GATK best practices for germline variant discovery.[12] Briefly, we aligned the reads to the macaque (Macaca mulatta) reference genome (Mmul_10, RefSeq: GCF_003339765.1), filtered out PCR-duplicates with Picard/2.23.4 and called variants with GATK/4.2 HaplotypeCaller and GenotypeGVCFs, set to output also invariant sites. Variants were filtered with GATK VariantFiltration using the recommended exclusion criteria (QD < 2.0, QUAL < 30.0, SOR > 3.0, FS > 60.0, MQ < 40.0, MQRankSum < -12.5, ReadPosRankSum < -8.0). We also removed sites where read coverage was less than half or more than twice the genome wide average, and masked repetitive regions following the SNPable regions pipeline (https://lh3lh3.users.sourceforge.net/snpable.shtml). Filtered autosomal variant calls (including invariant sites) were converted to fasta sequences and the PSMC-specific input format psmcfa using bcftools[11] and fq2psmcfa (https://github.com/lh3/psmc), respectively.

High-quality human data (Samples: HGDP00551, HGDP00931) (Figures S1F and S1G) was downloaded from the 1000 human genomes project (https://ftp.1000genomes.ebi.ac.uk/vol1/ftp/data_collections/HGDP/data/) and.cram files were converted to.bam files using samtools v1.14.[11] Subsequently, data was processed identically to the turtle dataset.

Historical population size inference

We ran PSMC with the settings "-N25 -t15 -r5", varying the parameter patterns (-p) between "4+25*2+4+6", "2+2+25*2+4+6" and, if needed, "1+1+1+1+25*2+4+6". To further test the effect of more parameters in deeper time intervals, we ran a broad range of parameters including up to 164 time intervals on simulated and real data (see Figures S1D and S1E). We used splitfa from the psmc-utils to split the autosomal psmcfa into shorter segments from which bootstrap replicates were subsequently sampled. Twenty and one hundred bootstrap replicates were run for primates and turtles, respectively. To avoid overfitting, we manually confirmed that at least 10 recombination events were inferred for each time window after 20 iterations. The only exception to this is when we specifically tested the effect of potential overfitting by setting parameters to "-p 2+2+16*2+4+6" on *P. unifilis* (Figure S1E). For turtles, PSMC inferences were scaled using a mutation rate of 7.9×10^{-9},[14,15] and generation times of 15 years for *Emydura subglobosa* and *Pelusios castaneus*, 25 years for *Podocnemis unifilis*, *Hydromedusa tectifera* and *Mesoclemmys tuberculata*, and 50 years for *Podocnemis expansa*. The primate PSMC inferences were scaled using a mutation rate of 4.91×10^{-9} and a generation time of 10 years for *Allochrocebus solatus* and *A. preussi*, and a mutation rate of 4.82×10^{-9} and generation times of 8 and 12 years for *Cercopithecus cephus* and *C. ascanius*, respectively.[9] Human inferences were scaled using a mutation rate of 1.25×10^{-8} and a generation time of 25 years. For Yoruba, the last two time intervals were merged to achieve enough recombination events per time window (see Figure S1).

Beta-PSMC extends the original PSMC model by allowing the population to vary within each discretized time interval.[10] As this improves resolution, especially in recent times, we explored whether Beta-PSMC was less prone to produce erroneous Ne spikes. Following the authors recommendations, we ran Beta-PSMC only with 20 parameter vectors spanning one discretized time interval (-p 20*1).

Genome simulation

We used "psmc2history.pl" and "history2ms.pl" to convert the PSMC outputs to an ms command. For each analysis we then simulated genomes (30 replicates) with 10 pairs of 30 Mb chromosomes using coalescent simulation with recombination.[13] First, we simulated a population that followed the exact inferred population history of the turtle species with the most pronounced peak in our data: *Podocnemis unifilis* to test whether PSMC could infer such a pronounced peak if it truly occurred. To test whether an increase in population structure, not size, could explain the extreme peaks, we simulated the same population history, but but kept the population size constant before it declines. Instead of the inferred peak in *P. unifilis*, we fragmented the previously panmictic population into three subpopulations with effective migration rates ranging from low (M=0.01) to high (M=10) (Figure S1). Following the observation that splitting the first time window removes the peak in our data, we finally simulated 30 genomes from a population with the demographic history inferred for *P. unifilis* with split parameter settings.

Published peak examples

We carried out a non-exhaustive search for published PSMC (and related methods like MSMC2) results with peaks in the second time interval, followed by a population collapse to very low Ne in the first time interval. For examples of studies reporting such results, see Table S1.

QUANTIFICATION AND STATISTICAL ANALYSIS

All necessary information on analyses carried out in this study are detailed in the method details.

ADDITIONAL RESOURCES

This study has not generated or contributed to a new website/forum and it is not part of a clinical trial.