

Genetic data suggest a natural prehuman origin of open habitats in northern Madagascar and question the deforestation narrative in this region

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The impact of climate change and anthropogenic deforestation on biodiversity is of growing concern worldwide. Disentangling how past anthropogenic and natural factors contributed to current biome distribution is thus a crucial issue to understand their complex interactions on wider time scales and to improve predictions and conservation strategies. This is particularly important in biodiversity hotspots, such as Madagascar, dominated by large open habitats whose origins are increasingly debated. Although a dominant narrative argues that Madagascar was originally entirely covered by woodlands, which were destroyed by humans, a number of recent studies have suggested that past climatic fluctuations played a major role in shaping current biome distributions well before humans arrived. Here, we address the question of the origin of open habitats in the Daraina region in northern Madagascar, using a multiproxy approach combining population genetics modeling and remote-sensing analyses. We show that (i) contrary to most regions of Madagascar, the forest cover in Daraina remained remarkably stable over the past 60 y, and (ii) the golden-crowned sifaka (*Propithecus tattersalli*), a forest-dwelling lemur, underwent a strong population contraction before the arrival of the first humans, hence excluding an anthropogenic cause. Prehuman Holocene droughts may have led to a significant increase of grasslands and a reduction in the species' habitat. This contradicts the prevailing narrative that land cover changes are necessarily anthropogenic in Madagascar but does not preclude the later role played by humans in other regions in which recent lemur bottlenecks have been observed.

habitat loss | primate | population bottleneck | coalescent

Over the past decades, loss and fragmentation of tropical forests have greatly increased at a global scale. Their impact on climate and biodiversity are of growing concern worldwide (1–4). Deforestation alone has been estimated to be the third most important source of carbon emissions, accounting for some 18–20% of global emissions (5). Madagascar is considered as one of the “hottest” biodiversity hotspots because of its high level of endemism and species richness (3, 6). The island is also reputed to be one of the most degraded tropical landscapes on Earth. Given that probably more than 90% of the Malagasy animal species live exclusively in forests and woodlands (7), the consequences of deforestation on biodiversity will be disastrous. Indeed, a recent study estimated that deforestation alone caused the loss of 9% of Malagasy species between 1950 and 2000 (8).

Human pauperization, economic activities, and population growth are thought to be the main causes for the degradation of natural habitats in Madagascar. In particular, the supposed great increase in deforestation rates over the past century is thought to have resulted from both the rapid population growth, from less than 3 million in 1900 (9) to 20.7 million in 2011 (10), and the use of fire for slash and burn cultivation (*tavy*) and cattle raising (11, 12). For instance, it has been estimated that during the past 50 y, 50–53% of the evergreen forests from eastern Madagascar

(13, 14) and 40% of the western deciduous forests have been cut down (14). Today, open habitats dominate vast areas in the center, west, and north of Madagascar, covering 80–84% of the island surface (14, 15). Colonial botanists (16, 17) who described Malagasy flora thought that Madagascar was a land entirely covered by woodlands when humans arrived and that the first humans were responsible for most of the deforestation across the island. However, earlier naturalists and travelers, such as Grandidier (18) or Gautier (19), defended a different view, namely, that the lack of forest in some regions was natural. Despite this early controversy, the “island-wide forest” hypothesis assuming the anthropogenic origin of grassy biomes remained pervasive during the 20th century (20–23) and continues to be largely used to justify conservation fundraising and management actions (24).

However, in the past few years, the question of the antiquity of these grassy biomes has become the center of a renewed controversy (15, 24–27). Indeed, a number of palynological and biogeographical records from various bioclimatic zones in Madagascar (27–32) have provided a mounting body of evidence suggesting that significant parts of the island may have been covered by a mosaic of forests, scrublands, and grasslands well before the earliest human settlements started to be demographically significant (i.e., 1,000 y ago) (11) and even before the earliest evidence of human arrival 2300 calibrated calendar years before present (Cal. YBP) (29). Directly related to this controversy, there is a hot debate over climatic vs. human causation (or both) of giant lemur and associated megafaunal extinction. Indeed, an increasing body of data has shown that intense hunting probably played a major role in the final demise of large-bodied mammals in Madagascar and elsewhere (29, 33–35). The elimination or reduction of this megafauna then contributed to major changes in fire regime (30) and the impoverishment of habitat diversity previously maintained by large seed dispersers (36–38). This “prehistoric overkill” scenario is well supported in regions like tropical Australia (39) or New Zealand (40), where megafaunal extinction occurred at a time of only slight climate and vegetation change. However, in Madagascar, the situation is less clear because most major climatic events of the late quaternary (i.e., droughts lasting millennia) (27, 31) predated human arrival. Also, the peopling of Madagascar was very uneven from the start, and different regions may thus exhibit different patterns and histories (11). We cannot therefore exclude that prehuman climate change triggered the shrinkage of forest habitats and the collapse of associated fauna as has been shown in other parts of the world (41–43). One way of addressing these issues is to determine whether species from different regions have been

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submitted to different demographic histories with different time depths. Indeed, the genome retains the specific signature of major events, such as recent contractions (44, 45) or ancient expansions (46). Reconstructing the demographic history of extant species may thus help to investigate how climate change and anthropogenic impact affected the dynamic of their habitat (42, 47).

In this study, we address one of the long-lasting and controversial questions of conservation biology, namely, the relative contributions of past anthropogenic and natural factors on the degradation of tropical ecosystems. We use an innovative multiproxy approach combining genetic, paleoclimatic, and remote-sensing data to question the origin and age of large open habitats in the region of Daraina in the north of Madagascar (Sava region). Forest habitat in this area corresponds to highly diversified forest fragments surrounded by a matrix of large grasslands and savannas. To assess the importance and dynamics of deforestation in the Daraina region, we combined aerial photography and satellite imagery to examine trends in forest cover change in the past 50 y, and thus to assess the importance of recent anthropogenic deforestation in Daraina compared with other regions (i.e., during the period of highest human density and impact). The genetic data were used to reconstruct the demographic history of the golden-crowned sifaka (*Propithecus tattersalli*), an endangered species of forest-dwelling lemur now restricted to the small region of Daraina, as a proxy for major changes in forest cover. Specifically, we tested the four following hypotheses: (H1) Open habitats in Daraina are recent and attributable to deforestation and human demographic growth since the first contact with Europeans (time period 1500–2000 A.D.), (H2) these open habitats are anthropogenic and dated from the first centuries of human settlement in the Daraina region (1000–1500 A.D.), (H3) open habitats were human-induced and dated from the first millennium of human presence on the island (2300–1000 Cal. YBP), and (H4) open habitats are mainly natural and expanded in relation to Holocene climatic fluctuations in prehuman times (10,000–2300 Cal. YBP).

Our results show that (i) the forest cover in the Daraina region has remained remarkably stable over the past 50 y, exhibiting very low rates of deforestation in comparison to other regions; (ii) the golden-crowned sifaka genetic data exhibit a clear and robust signal of a population contraction; (iii) the dating of this collapse is anterior to the arrival of the first humans, thus excluding an anthropogenic cause (hypothesis H1, H2, or H3); (iv) the population contraction was most likely induced by climatic events, such as late prehuman Holocene droughts leading to significant shifts in

vegetation in northern Madagascar and modification in *P. tattersalli*'s habitat and resource availability; and (v) in this region, most present-day open habitats are not attributable to recent human activities but most likely originated from climate changes occurring several millennia ago. These results suggest that a reappraisal of the representation of the impact of local communities on their environment may be necessary among conservation biologists.

Results

Trends in Forest Cover Change in the Daraina Region. For the recent period (1949–2002), diachronic analyses of different image sources and maps (Landsat images taken in 1972, 1994, and 2002; 1/100,000 topographical map, 1960; aerial photography, 1949) show a relative global stability of the forest cover surface in the distribution area of *P. tattersalli* (Fig. 1). In most cases, the main forest blocks have kept their integrity, and even seem to have grown, but the situation is locally contrasted. Deforestation dominates on the western part of the area, whereas forest progressed on wide sections of the east and southeast. Because of these opposite dynamics and the differences in the spatial and spectral resolutions of the sensors and data sources, quantifying the global forest evolution of the region or a fragmentation index would be hazardous. However, field investigations (i.e., interviews of old farmers) and a focus on two zones (Fig. S1) confirm this two-way evolution. The driest semideciduous forest of the northwestern part of the region has been affected by a progressive deforestation, particularly on the western hillsides, whereas the eastern massifs not only appear intact but significantly progressed during the period.

Demographic History of Endemic Golden-Crowned Sifakas. We found evidence of a population collapse for all *P. tattersalli* samples across all forest fragments (Fig. S2A and B). The signal was robust to the sampling strategies and subpopulations considered (Fig. S3). Across the different analyses, we found that the effective population size of *P. tattersalli* decreased by one to two orders of magnitude (Fig. S2B) from an ancestral population N_I with a median ranging from 21,715–30,915 to a present-day effective population size N_0 with a median between 430 and 795 (depending on the genetic unit analyzed: K_A , K_B , or the global sample). The posterior distribution of T , the time when the population started to decrease, exhibited median values that were older than the arrival of humans in Madagascar, with medians ranging from 7,183 to 7,567 Cal. YBP among samples (assuming a generation time of 6 y) (Fig. S2C). The Bayesian

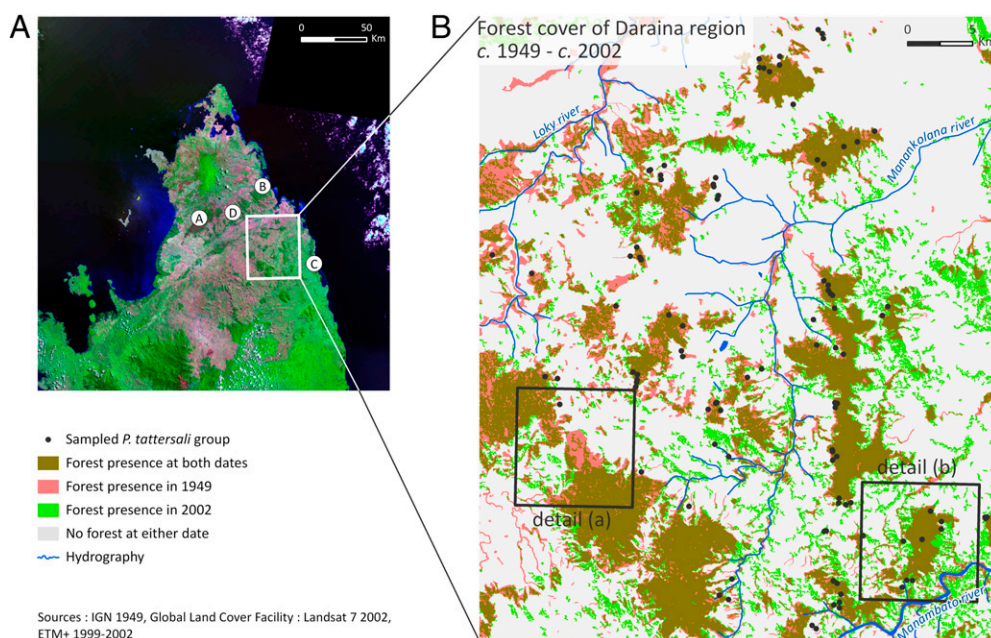


Fig. 1. Trends in forest cover change in the Daraina region between 1949 and 2002. (A) Circled letters on the satellite imagery of the north of Madagascar indicate the locations of the four places named in the main text and in Fig. 4: Ankarana massif (A), Irodo (B), Vohémar (C), and Andavakoera (D). (B) Black dots on the map of the Daraina region show the sampling locations of the 105 *P. tattersalli* social groups genotyped. This figure shows that trends in forest cover change radically in the northwestern (deforestation) and southeastern (forest regeneration) parts of the region as exemplified by the two focus zones [detail (a) and detail (b)] for the periods 1949–1972, 1972–1994, and 1994–2002. The two focus zones are detailed in [Fig. S1](#).

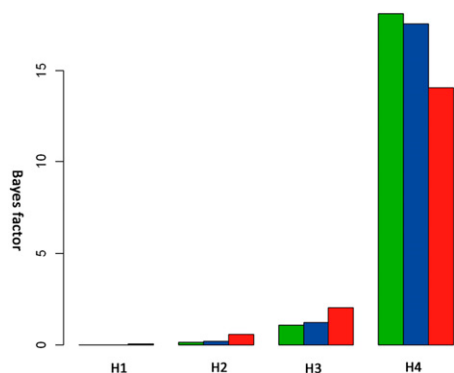


Fig. 2. BF values for the four tested scenarios under the different sampling schemes. H1 corresponds to a contraction attributable to anthropogenic effects following the arrival of Europeans (0–500 Cal. YBP); H2 (500–1,000 Cal. YBP) corresponds to a contraction attributable to anthropogenic effects during the period for which first cities appeared in far northern Madagascar; H3 (1,000–2,000 Cal. YBP) corresponds to the first millennium of human presence, during which human densities were probably very low; and H4 (2,000–10,000 Cal. YBP) corresponds to a contraction caused by environmental factors before the arrival of humans in Madagascar. In the “global sample” analyses (green), samples were taken across the whole sampled area, ignoring any substructure identified in a previous study. In the K_A (red) and K_B (blue) analyses, the samples were taken only considering the K_A and K_B genetic clusters identified in a previous study (details are provided in *Materials and Methods*).

method that we utilized uses lognormal priors for T , which may favor recent over ancient times. The Bayes factor (BF) analysis, which uses ratios of posteriors over priors for comparing competing hypotheses, allows one to circumvent this potential problem. The BF analysis (Fig. 2) clearly favored the fourth scenario with extremely high values (H4, $BF > 14$ –19) over the

other scenarios that assumed an anthropogenic cause for the contraction of *P. tattersalli* populations ($1 < BF < 3$). The BF analysis was robust to changes in generation length, even when we used an unrealistically low value of 3 y (H4, $BF > 18$) (Table S1), hence indicating that golden-crowned sifaka populations contracted before humans arrived on the island. Furthermore, the same pattern of results was obtained when we computed BFs for equal length time periods of 500 y between the present and 50,000 y ago (Fig. 3A) or 10,000 y ago (Fig. 3B). The highest BF values ($BF > 9$) correspond to a time period ranging from 5,500–10,000 y ago, whereas the lowest values were observed for the last couple of millennia ($BF < 2$).

Discussion

During the past centuries, industrialized agriculture, forestry, and rapid urban and rural population growth have led to the global homogenization of landscapes at the continent scale and to the mass extinction of thousands of species worldwide (1, 48). The suddenness and extent of these changes have led several authors to identify this extremely short period as the Anthropocene. We cannot ignore the major impacts of humans on natural ecosystems (39, 49, 50). Indeed, there is an ample fossil record illustrating the preponderant role of human hunting on the extinction of native megafauna in Madagascar (30, 33) and other parts of the world (51). Furthermore, it is now generally accepted that megafaunal extinction triggered a large change in fire regime, affecting the structure, composition, and dynamics of plant communities and favoring more dense, uniform, and impoverished formations (30, 38, 39). However, the millennia preceding the Anthropocene should not be neglected either. Indeed, increasing data are pointing to the late Pleistocene and the Holocene as major “reshuffling” periods during which many species saw their ranges contract or shift, whereas others conquered new territories (52–54). Late Quaternary climate changes thus contributed to the extinction of large-bodied species (42, 55) both in temperate (56, 57) and tropical regions, including Madagascar (58–60).

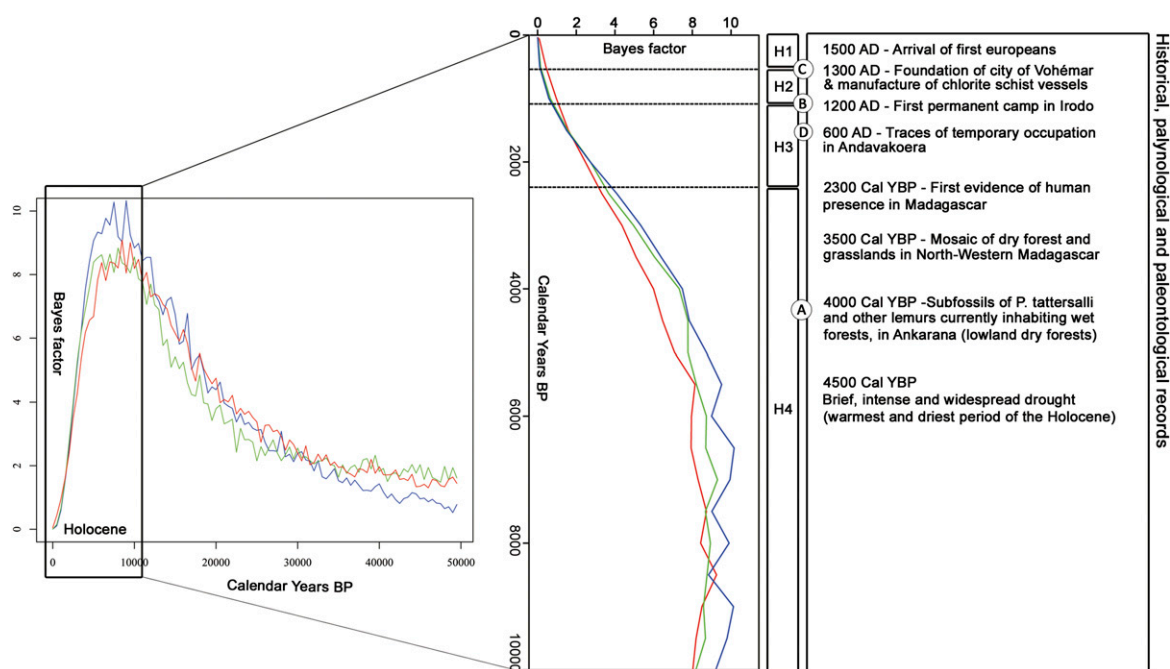


Fig. 3. Most likely period for the start of the population decrease. This figure shows the value of the BF for fixed-length intervals of 500 y between 0 and 50,000 y ago and between 0 and 10,000 y ago (i.e., the Holocene period). The BF measures the weight of evidence for the hypothesis that the population started to decrease in a given interval (H_A) vs. any other time period (H_B). The different solid lines correspond to different sampling strategies described in *Materials and Methods*: red (genetic unit K_A), blue (genetic unit K_B), and green (global sample). $BF = 1$ indicates that H_A and H_B are equally likely. BF values greater (or smaller) than 1 support H_A (or H_B). We considered BF values > 7 as significant evidence for H_A . We indicated, along the time frame, the intervals corresponding to the four tested scenarios (H1–H4) and the historical, palynological, and paleontological evidence discussed in the main text.

In this study, we add several independent pieces to the puzzle of the present location and paleodistribution of *P. tattersalli* and shed light on the origin of their habitat loss and fragmentation. Our genetic data and corresponding Bayesian analyses suggest that the golden-crowned sifaka underwent a drastic contraction during the Holocene, most likely between 5,500 and 10,000 y ago. This event certainly took place well before the earliest evidence of human presence in Madagascar (2300 Cal. YBP), which is itself earlier than the first traces left by humans on their environment, around 1,000 y ago (11). The reduction of *P. tattersalli*'s range and population size is most likely attributable to climatic fluctuations (31, 61–63). Several widespread and pronounced droughts have been documented during the Holocene in Madagascar (27) and in eastern Africa (54, 64). For instance Virah-Sawmy et al. (27) documented how an open woodland transformed into a grassland between 5,800 and 5,200 y ago in southern Madagascar. The same drought probably caused similar shifts from humid and continuous woodland forests to savanna-type habitats in many regions of the island, including the Daraina region. MacPhee et al. (63) and Matsumoto and Burney (32) also suggested that grasslands were already common in the “dry deciduous forest” ecoregion of the western coast well before any evidence of anthropogenic deforestation. Several recent studies have also argued for the relative antiquity of western grasslands on the basis of the large diversity of C_4 grass lineages and the presence of plant and animal species endemic to Malagasy grassy biomes (15, 65).

Our remote-sensing analyses showed that in the Daraina region, the forest cover remained remarkably stable during the past 60 y, exhibiting a very low rate of deforestation in comparison to other regions (66). Indeed, deforestation has been only observed at very local scales and was counterbalanced by the numerous areas where regrowth occurred. This may appear surprising compared with common narratives and most scientific papers (13, 14), but a wide majority of studies on the evolution of Madagascar forests only considered the disappearance of a supposed pristine and static forest. This one-way analysis omits to consider the possible forest regeneration and the forest as a living and complex ecosystem that is not necessarily destroyed by human activities (24, 25). This is not an isolated case in Madagascar, and some recent studies also point to a possible forest resilience to (human or natural) disturbances and even forest regeneration (67, 68). This unexpected small change in forest cover during the period of highest population density is yet another argument suggesting that human impact was probably limited during the period of human occupation and that present-day open habitats are, in some regions at least, the result of ancient climatic changes. Nevertheless conservation management seems to find it hard to accept or even take into account this point of view, even though evidence of the ancientness of Madagascar's grassy and savannah lands is increasingly well-documented (15, 28, 32, 69).

As a result of differences in preservation potential across regions and research efforts, the paleontological data are not equally distributed across Madagascar (70). Subfossil localities are rare in the north of the island and provide limited information on the past distribution of extant species. However, subfossils of *Haplemur simus* (dated to $4,560 \pm 70$ Cal. YBP) (71) and *Indri indri* (dated to $4,400 \pm 60$ Cal. YBP) (72), two lemur species currently living exclusively in limited areas of the eastern evergreen humid forests of Madagascar, have been found in the Ankarana massif cave system, just northwest of the Daraina region (Fig. 1A). This suggests that the paleodistribution of these two species extended considerably beyond their present range. On the basis of these data alone, we cannot establish the timing and cause of their range contraction; however, we can assume a role of climate aridification because the Ankarana massif is now classified among the driest regions of the island (71–73). Interestingly, despite a poor fossil record, bones attributed to *P. tattersalli* were also found in the Ankarana cave system (72, 73), suggesting that the paleodistribution of the golden-crowned sifaka was also much wider than today. The hypothesis that *P. tattersalli* saw its distribution contract as a result of climatic rather than anthropogenic changes is also supported by phylogeographic data. Indeed, *P. tattersalli* has

a disjunct and restricted distribution in the northeastern part of the island far removed from the northern limit of its sister species, *Propithecus coquereli*. Unlike *P. tattersalli*, *P. coquereli*, like the other related species *Propithecus deckeni*, *Propithecus coronatus*, and *Propithecus verreauxi*, present wide, parapatric, and contiguous ranges along the west coast (74) (Fig. S4). An ancient contraction of *P. tattersalli* would thus explain both fossil and present-day distribution data.

Another set of arguments against a major human impact on *P. tattersalli*'s forest habitat comes from the available data on human occupation records in northern Madagascar. These data indicate that until the late 19th century, human populations were sparsely distributed in the north, with primarily fishermen and pastoralists occupying small coastal villages (11). Clear evidence of permanent occupation was detected only in the 12th century in Irodo (11, 75) (Fig. 1A). Furthermore, the largest city of the region, Vohémar (Fig. 1A), only appeared around 1350 A.D. (75). It was known to Europeans in the 16th century but was largely abandoned by the late 1700s (11). At the end of the 19th century, the total population was less than 1,800 (76). Today, the area remains poorly inhabited, with fewer than 30,000 people and a density of less than 12 individuals per square kilometer (ind/km^2) (77) compared with an average of 33 ind/km^2 across Madagascar and above 60 ind/km^2 in the central highlands (78), where forests are absent to a large extent. Local Daraina populations primarily depend on subsistence economy dominated by shifting and irrigated agriculture, cattle raising, extraction of forest products, and fishing (77, 79).

Conclusion

There is no doubt that deforestation has been a major phenomenon during the past century in many regions of Madagascar (14, 66) and that humans played a major role in driving numerous species to extinction since they arrived in Madagascar. This is particularly true for the megafauna. However, our work contradicts the prevailing narrative that land cover changes are necessarily anthropogenically induced and negative across the whole island. Indeed, even if *P. tattersalli*'s population size and range were decreasing today, the genetic data appear to keep memory of a much older event or series of events, in which humans cannot have played a role. This result does not necessarily hold across all of Madagascar. For instance, one of us (L.C.) has contributed to the demonstration that severe and very recent population declines affected mouse lemurs [*Microcebus* spp. (45)] and sportive lemurs [*Lepilemur* spp. (44)] from the northwest of Madagascar, where habitat loss has been massive in the past century (32). Similarly Lawler (80) concluded that *P. verreauxi* also suffered from a bottleneck less than 2,000 y ago in the southwest of Madagascar, again, a region where human impact and deforestation are more serious than in Daraina (14). Similar results have also been suggested for other primates [orangutans (81) and howler monkeys (82)]. In all these species [detection of a more ancient population size change is discussed elsewhere (46, 47)], the population bottleneck has been reasonably attributed to recent human-driven changes.

Overall, the patterns of extinction of the world's terrestrial megafauna are best explained by models combining human arrival and climatic variables (35, 41–43). However, the relative contribution of both factors appears to vary strongly among regions (53, 83–85) in relation to the velocity of climate changes (55, 86) and the pattern of human settlement and activities (35, 39). Furthermore, each species appears to respond differently to the effects of climate change, habitat redistribution, and human encroachment (42, 87) in relation to their taxonomic group (85) as well as their body size and ecological traits (53, 83). In the case of *P. tattersalli*, we did not detect recent population collapse, probably because this species is protected by a local taboo (*fady*) and is not normally hunted. However, our aim here is not to be overly optimistic. The species range is among the most restricted of any lemurs (74), and the recent political crisis that began in 2009 has led to the increase of poaching by people originating from other regions that do not have the same *fady*s. The whole Daraina region may also be strongly affected in the near future by the pending tarring of the main road bisecting the

species range. However, it is important that conservation actions, particularly from international institutions that cannot always account for regional differences, should not consider human proximity only as a threat and should not base their action on diagnoses pointing to environmental degradation by traditional land management because we saw that forest regeneration occurred in a context of human occupation. It seems risky to alienate the local communities by excluding them from their territories (88), whereas they can be precious allies to help conservationists find adapted local answers for sustainable natural resources management.

Our study calls for more regional and comparative studies that will help reconcile paleontological, genetic, and potentially geological data. More work is also needed to apprehend the history of regions that have experienced various composite histories in term of landscape transformation, human activities, and cultural attitudes toward their environment (11, 84). Multidisciplinary approaches that integrate information from genetic, paleoclimatic, biogeographic, and remote-sensing data, together with contributions from anthropology, sociology, and political sciences to clarify the relative contributions of human and natural factors on the past and ongoing environmental changes, are urgently needed in the face of major changes to come.

Materials and Methods

A short version of the materials and methods used in this study is provided in this section. Details are provided in *SI Materials and Methods*.

Golden-Crowned Sifaka (*P. tattersalli*). The golden-crowned sifaka (89) is a medium-sized endangered lemur species only found in the Daraina region, located between the Loky and the Manambato Rivers on the northeastern coast of the SAVA region. Its geographic range is highly fragmented and one of the most restricted of any lemurs (74, 90).

Analysis of Forest Cover Changes in the Daraina Region During the Period 1949–2002. To analyze the recent trends in forest changes in the Daraina region, we first used three Landsat images taken in 1972 (November 9), 1994 (September 2), and 2002 (May 27). Supervised classification was performed on three bands (Normalized Difference Vegetation Index, Green, and Near Infra-Red) on each scene using the ENVI algorithm Support Vector Machine (ITT). Classifications were validated using field knowledge and very high-resolution data for part of the area (SPOT imagery, QuickBird Imagery from GoogleEarth). Additionally, we used the topographical maps from Foiben-Taosarintanin'i Madagasikara (FTM, Malagasy Geographical Institute) based on aerial images from 1949 to analyze the trends in forest cover changes for the period 1949–1972. These maps were georeferenced, and their forest zones were manually digitized. No information was available on the methods used by the FTM to create topographical maps from aerial images. We thus interpret the results of this latter analysis with caution and by considering the global patterns of change across these two dates qualitatively rather than quantitatively.

***P. tattersalli* Sample Collection and DNA Analysis.** Fecal material from 292 putative individuals belonging to 107 social groups was collected in the nine main forest fragments of the species range during two field missions in 2006

and 2008. Extraction and genotyping procedures were performed as described by Quéméré et al. (91). DNA was amplified using a set of 13 (or 20) micro-satellite loci. We obtained reliable multilocus genotypes for 230 unique individuals (105 groups) (92). We performed the demographic history analyses detailed below by considering the whole sample (i.e., 230 individuals from the entire species range) or the individuals from K_A ($n = 83$ individuals) and K_B ($n = 126$ individuals) genetic units separately (*SI Materials and Methods*).

Past Demography Analysis. To investigate the demographic history of *P. tattersalli*, we used a full-likelihood Bayesian inferential approach designed to detect, quantify, and date changes in population effective size (93). This method was originally developed by Beaumont (94) and implemented in the program MSVAR 1.3 (93). The underlying model assumes that a population of size N_t (ancestral population size) started to decrease (or increase) exponentially T generations ago to the present-day population size (N_0). Mutations are assumed to occur under the Stepwise Mutation Model (SMM) at a rate $\theta = 2N_0\mu$, where μ is the per locus mutation rate per generation. MSVAR estimates the posterior distribution of the model parameters (N_0 , N_t , t , μ), given the allelic distribution and prior information, using Markov chain Monte Carlo simulations. For each dataset, we performed four independent runs with wide uninformative priors (Table S2) and three different values of generation length (3, 6, and 17.5 y; justification is provided in *SI Materials and Methods*). To compare alternative scenarios and identify the most likely scenario within a Bayesian framework, we performed a BF analysis (e.g., ref. 82). A BF of 1 indicates that the two hypotheses are equally probable, whereas values greater than 1 would favor H1 and values less than 1 would favor H2. BFs greater than 4 are considered as positive evidence for H1, and BFs greater than 7 are considered as significant evidence for H1 (e.g., 93). We identified four time intervals corresponding to four scenarios indirectly related to the origin of grasslands in the Daraina region. The BFs were first computed for each of the four time intervals against all other periods taken together. Given that the different scenarios corresponded to time periods of different duration, we additionally computed BFs for equal length intervals covering the whole Holocene time period (i.e., 10,000 y) and the past 50,000 y.

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Supporting Information

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SI Materials and Methods

Study Site: Daraina Region. The Daraina area (250,000 ha) is located between the Loky and Manambato Rivers in the Tsaratanana hydrogeographic basin on the northeastern coast of the SAVA region. It is covered by a highly heterogeneous matrix of grasslands, dry scrub, agricultural lands, zebu cattle-grazing pastures, and forest fragments connected by an extensive network of gallery forests (1). Although most of the forest habitat corresponds to lowland dry deciduous formations similar in composition to dry western Malagasy forests, the region also contains humid forests, semievergreen forests, and premontane evergreen forests covering hills and mountain tops, respectively (2). Despite its severe fragmentation, the region exhibits a high level of biological diversity. It has been designated as a high priority area for biodiversity conservation in Madagascar (3), and some of the forest fragments were designed in 2005 as a newly protected area of the Madagascar's Protected Areas System (Système d'Aires Protégées de Madagascar) to minimize direct anthropogenic pressures, such as intensive wood and mining exploitations.

Study Species: Golden-Crowned Sifaka. The golden-crowned sifaka (*Propithecus tattersalli*) is a medium-sized endangered lemur species only found in the Daraina region. Its geographic range is fragmented and one of the most restricted of any lemurs (4). Like other species of sifaka, *P. tattersalli* forms social groups averaging between three and four individuals per group, with a range between two and nine. A previous genetic study has shown that the species maintains high diversity within forest fragments and dispersal among most forest fragments (5). These investigators have also shown that the major environmental feature structuring genetic diversity is the Manankolana River, which defined two main genetic clusters. The eastern riverside cluster was named K_A . The western cluster could itself be subdivided into two clusters, K_B and K_C , with the latter being located in the northern part of the western riverside. All clusters included several forest fragments, hence suggesting high gene flow among these fragments, particularly for K_A and K_B .

Analysis of Forest Cover Changes in the Daraina Region During the 1949–2002 Period. To analyze the recent trends in forest changes in the Daraina region, we first used three Landsat images taken in 1972 (November 9), 1994 (September 2), and 2002 (May 27). All three images are orthorectified. The scene from 1972 is Landsat 1 Multi-Spectral Scanner (MSS) with a 57-m resolution, and scenes from 1994 and 2002 are, respectively, Landsat 5 Thematic Mapper (TM) and Landsat 7 TM, both with a 28.5-m resolution. The images from 1972 and 1994 were taken during the end of the dry season, whereas the image from 2002 was taken during the beginning of the dry season. ENVI 4.4 software was used for image analysis operations, and ArcGIS 9.3 software was used for spatial analysis operations. The scenes from 1994 and 2002 were resampled using cubic convolution with a 57-m resolution to allow us to compare them with the 1972 scene. To avoid classifying the whole Landsat scene, a subscene was made from each scene to the area of interest. This area is roughly $51.5 \times 61 \text{ km} = 3,142 \text{ km}^2$ and is located between latitudes $-13^\circ 20 \text{ min}$ and $-12^\circ 52 \text{ min}$ and between longitudes $49^\circ 23 \text{ min}$ and $49^\circ 56 \text{ min } 30 \text{ s}$. Images from 1994 and 2002 were found to superpose correctly and did not need any georeferencing. The 1972 scene was georeferenced on the 1994 scene with a total RMS error of 0.4. Clouds from each scene were digitized and used to create a mask, which was then applied during each classification to

avoid taking these zones into account. A vegetation index was calculated for each scene. We used the Normalized Difference Vegetation Index (NDVI), calculated as follows: $(\text{Near Infra Red} - \text{Red})/(\text{NIR} + \text{Red})$, where NIR is MSS3 for the 1972 scene and TM4 for 1994 and 2002 scenes. Supervised classification was performed on three bands (NDVI, Green, and NIR) of each scene using the ENVI algorithm Support Vector Machine (ITT). This combination of bands was used to remove shade effects, with the area of interest being in a hilly landscape. Seven classes were used, and only the two classes corresponding to forest were retained from the classified images for further analysis. Classifications were validated using field knowledge and very high resolution data for part of the area (SPOT imagery, QuickBird Imagery from GoogleEarth). Additionally, we used the topographical maps from Foiben-Taosarintanin'i Madagasikara (FTM; Malagasy Geographical Institute) based on aerial images from 1949 to analyze the trends in forest cover changes for the period 1949–1972. These maps were georeferenced, and their forest zones were manually digitized in ArcGIS 9.3. For the two focus zones, we also used the original aerial photographs to control the validity of the process. Note that the calculation of the rate of forest change for this period should not be taken at face value because no information was available on the methods used by the FTM to create topographical maps from aerial images. We thus interpret the results of this latter analysis with caution and by considering the global patterns of changes during these two dates qualitatively rather than quantitatively.

Sample Collection and DNA Analysis. Fecal material from 292 putative individuals belonging to 107 social groups was collected in the nine main forest fragments of the species range during two field missions in 2006 and 2008. All the samples were obtained immediately after defecation, as described by Quéméré et al. (6). DNA extraction was carried out using the 2CTAB/2PCI approach described by Vallet et al. (7) in a class I microbiological safety hood to avoid contamination. DNA was amplified using a set of 13 microsatellite loci isolated from *P. tattersalli* (8 loci) and from the closely related (sister) species *Propithecus coquereli* (5 loci). A subsample of the data was also analyzed with 7 additional loci (total of 20 loci, details provided below). The multitube approach detailed by Frantz et al. (8) was used to ensure the reliability of genotypes. The extraction and genotyping procedures are detailed by Quéméré et al. (6). We obtained reliable multilocus genotypes for 230 unique individuals (105 groups). As noted above, a recent analysis of the *P. tattersalli* spatial genetic structure has shown that the species is divided into three main genetic units corresponding mainly to social groups living east (K_A) or west (K_B and K_C) of the Manankolana River, with the latter unit (K_C) corresponding to an isolated fragment called Antsaharaingy in the far north (5). We performed the demographic history analyses detailed below by considering the whole sample (i.e., 230 individuals from the entire species range) or the individuals from K_A ($n = 83$ individuals) and K_B ($n = 126$ individuals) units separately.

Past Demography Analysis. To investigate the demographic history of *P. tattersalli*, we used a full-likelihood Bayesian inferential approach designed to detect, quantify, and date changes in population effective size (9). This method was originally developed by Beaumont (10), and the improved hierarchical method was implemented in the program MSVAR 1.3 (9). It has been used with success on several species for which it helped to

identify, quantify, and date population size changes (11, 12). A recent simulation study has demonstrated the high efficiency of this approach to detect population size changes (13). That study showed it was particularly efficient at detecting both recent and ancient bottlenecks and provided much better results than any of the summary-statistics methods with which it was compared. The model underlying the MSVAR 1.3 program assumes that a population of size N_t (ancestral population size) started to decrease (or increase) exponentially T generations ago to the present-day population size (N_0). Mutations are assumed to occur under the Stepwise Mutation Model (SMM) at a rate $\theta = 2N_0\mu$, where μ is the per locus mutation rate per generation. MSVAR estimates the posterior distribution of the model parameters (N_0 , N_t , t , μ), given the allelic distribution and prior information, using Markov chain Monte Carlo (MCMC) simulations. For each dataset, we performed four independent runs with wide uninformative priors and different starting points as in the study by Olivieri et al. (14). Each analysis was run for 5×10^9 generations with samples taken every 100,000 steps after a 10% burnin. The details of the runs, including the prior distributions, are given in Table S2. To test for convergence of the MCMC chains, the convergence diagnostic statistic of Gelman and Rubin (15) and Brook and Gelman (16) was applied on datasets from the four consensus chains using the coda R package (17). To express time in years (T), we considered a generation length value of 6 y. The only data available in the literature on the age of sexual maturity and the age at first reproduction of sifakas have been collected for *Propithecus verreauxi*, a closely related species. Richard et al. (18) observed that fewer than half of the *P. verreauxi* females give birth for the first time by the age of 6 y and that the rate of successful reproduction (defined as infant survival for 12 mo) is very low before this age. The IUCN uses a value of 10 y for the generation time of *Propithecus coronatus*, *Propithecus deckeni*, and *P. verreauxi*. In a recent study, Lawler et al. (19) used multimodel inference (20) to estimate the demographic parameters (growth rate and reproductive rate) of a population of *P. verreauxi* and found that the generation time could be as long as 17.5 y. Although the latter figure seems counterintuitively high and may depend on several model assumptions made by these authors, it suggests, together with the other data, that the generation time of *P. tattersalli* is likely to be longer than 6 y. By using 6 y in our calculations, we are thus unlikely to overestimate the age of population size changes as we discuss below.

To compare alternative scenarios and identify the most likely scenario within a Bayesian framework, it is possible to perform

a Bayes Factor (BF) analysis (21). BFs assess the “weight of evidence” of alternative hypotheses, say of H_1 over H_2 , based on the data, D , by comparing the ratio of their posterior probabilities with the ratio of their prior densities. A BF of 1 indicates that the two hypotheses are equally probable, whereas values greater than 1 would favor H_1 and values less than 1 would favor H_2 . BFs between 1/3 and 3 are usually considered not worth mentioning, whereas BFs greater than 4 (smaller than 1/4) are considered as positive evidence for H_1 (and, respectively, for H_2) and BFs greater than 7 (smaller than 1/7) are considered significant evidence for H_1 (and, respectively, for H_2) (9). As noted in the main text, we identified four time intervals corresponding to four scenarios indirectly related to the origin of grasslands in the Daraina region. The BFs were first computed for each of the four time intervals against all others periods taken together. Given that the different scenarios corresponded to time periods of different duration, we additionally computed BFs for equal length intervals covering the whole Holocene time period (i.e., 10,000 y). We used periods of 500 y to have enough points to compare the priors and posteriors (22). Shorter time periods were also tested and provided the same trend but were more ragged because of stochastic variance in the estimation of the posteriors.

The model underlying MSVAR assumes that samples were obtained from isolated populations that can be approximated by a Wright–Fisher model, hence ignoring genetic substructure and migrations from other populations. Several recent simulation studies have demonstrated that population subdivision and/or isolation by distance can falsely generate a signature of population collapse (23–25). Chikhi et al. (23) have shown that the spurious bottleneck signal is particularly important when genetic diversity and differentiation are large. However, they have also shown that this spurious bottleneck disappears to a large extent when samples are taken from several populations or demes. To evaluate whether population structure is partly responsible for bottleneck signals detected in *P. tattersalli*, we performed additional runs varying our sampling strategy within the identified genetic clusters (K_A and K_B). We generated new samples of 20 individuals using several sampling strategies: (i) by taking individuals at random within a genetic cluster and (ii) by maximizing or (iii) by minimizing the number of individuals per social group within a cluster. To determine whether the results were robust to an increase in the number of loci, we genotyped the individuals from the eastern genetic cluster (K_A) using 7 additional loci (i.e., 20 loci in total). The results obtained with 20 loci were consistent with those observed with 13 loci.

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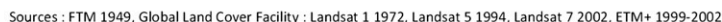


Fig. S1. Trends in forest cover change in the Bobankora (a) and Antsahabe (b) forests. This figure shows a detailed analysis of the evolution of the forest cover in the two focus zones shown in Fig. 1. Red and green areas correspond to deforested and reforested areas, respectively, for the corresponding time periods represented in each panel.

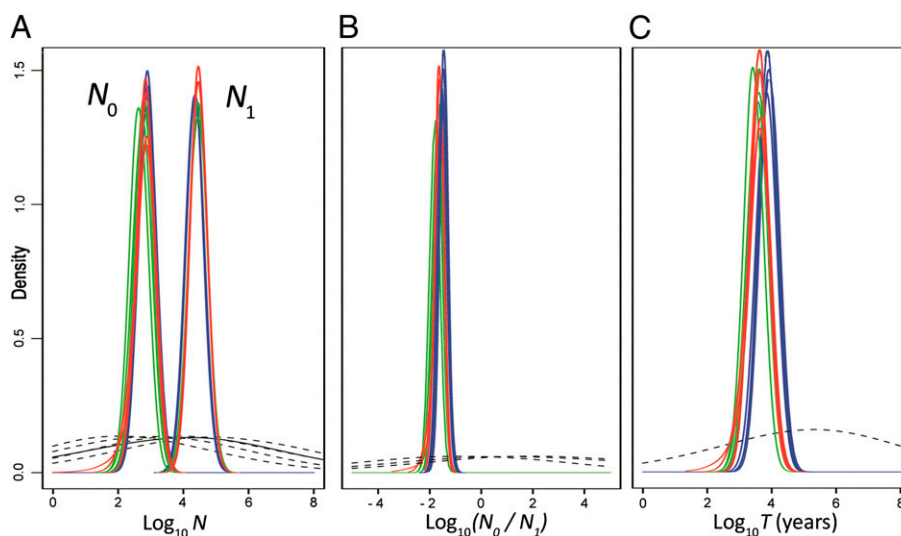


Fig. S2. Present and past population sizes, and time since the population size change. (A) Posterior distributions of present (N_0) and past (N_1) population sizes shown as solid lines: red (genetic unit K_A), blue (genetic unit K_B), and green (global sample). The dotted and dashed lines represent priors for N_0 and N_1 , respectively. (B) Ratio of N_0 to N_1 (in logarithmic scale). Negative values correspond to population contraction, whereas positive values correspond to population expansions. (C) Posterior distribution for the time since the population collapse. The posterior distributions have medians around 7,000 Cal. YBP (details are provided in the main text).

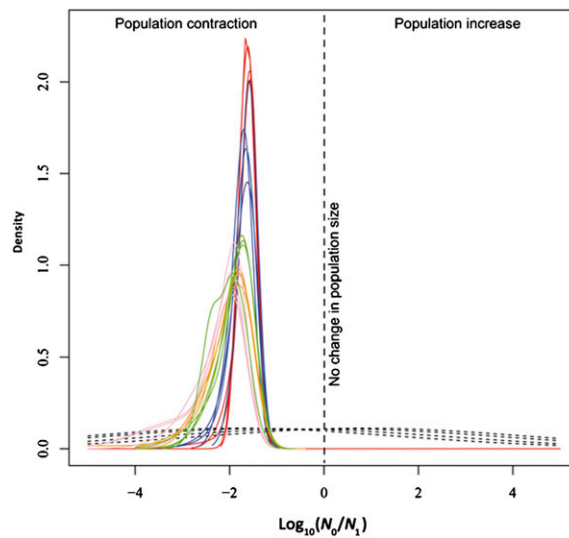


Fig. S3. Effect of the sampling scheme on the detection of population size changes. Ratio of N_0 to N_1 (in logarithmic scale). Negative values correspond to population contraction, whereas positive values correspond to population expansion. The results shown were obtained for the genetic unit K_A under different sampling schemes: blue lines (83 individuals, 13 loci), red lines (83 individuals, 20 loci), pink lines (20 individuals, minimizing the number of individuals per social groups), orange lines (20 individuals, maximizing the number of individuals per social groups), and green lines (20 individuals, random strategy). For each sampling scheme, we did four runs using the prior and hyperprior values indicated in Table S1. We observed a strong signal of population decline robust to the sampling strategies and subpopulations considered.

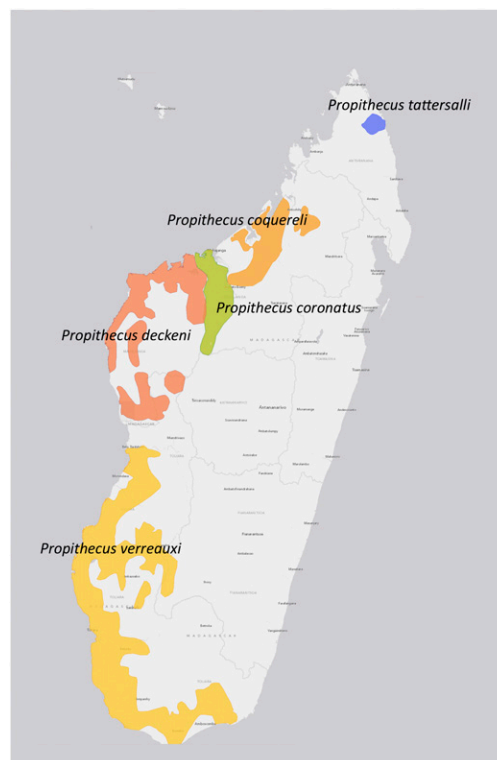


Fig. S4. Distribution of sifaka species of group “verreauxi” in northern Madagascar. *P. tattersalli* has a disjunct and restricted distribution in the far north-eastern of the island distant from the northern limit of its sister species, *P. coquereli*. Unlike *P. tattersalli*, *P. coquereli*, like other related species *P. deckeni*, *P. coronatus*, and *P. verreauxi*, presents wide, parapatric, and contiguous ranges along the west coast.

Table S1. BFs for H1–H4 using different generation length values

	G	H1 (0–500 y)	H2 (500–1,000 y)	H3 (1,000–2,000 y)	H4 (2,000–10,000 y)	H4' (10,000–50,000 y)
Global sample	6	0.00	0.12	1.08	18.10	5.06
	3	0.01	1.13	5.67	27.71	0.92
	17.5	0.00	0.00	0.02	1.92	26.98
Genetic unit K_B	6	0.00	0.17	1.21	17.53	5.11
	3	0.02	1.26	5.96	26.12	0.94
	17.5	0.00	0.00	0.04	2.00	25.62
Genetic unit K_A	6	0.03	0.56	2.00	14.04	4.98
	3	0.10	2.10	6.11	18.55	1.23
	17.5	0.01	0.12	0.26	2.69	17.54

This table shows the BFs for the four hypotheses discussed in the main text for different generation lengths. The value of 6 y was used throughout the main text and is probably a slight underestimation. The value of 17.5 y was recently suggested by Lawler et al. (19) for *P. verreauxi*, and it is likely to be an overestimation (*Materials and Methods*). The value of 3 y corresponds to the *P. verreauxi* female age of sexual maturation (18). Whichever value is used, the highest BF values are obtained for H4.

Table S2. Starting values for priors and hyperpriors for the four MSVAR 1.3 MCMC independent runs

Runs	Starting values for priors (mean, variance)				Hyperpriors			
	Log (N_0)	Log (N_1)	Log (θ)	Log (T)	Log (N_0)	Log (N_1)	Log (θ)	Log (T)
1	4, 1	5 , 1	–3.5, 1	5, 1	3, 2, 0, 0.5	4 , 3, 0, 0.5	–3.5, 0.25, 0, 0.5	5, 3, 0, 0.5
2	4, 1	4 , 1	–3.5, 1	5, 1	3, 2, 0, 0.5	3 , 3, 0, 0.5	–3.5, 0.25, 0, 0.5	5, 3, 0, 0.5
3	4, 1	3 , 1	–3.5, 1	5, 1	3, 2, 0, 0.5	2 , 3, 0, 0.5	–3.5, 0.25, 0, 0.5	5, 3, 0, 0.5
4	4, 1	2 , 1	–3.5, 1	5, 1	3, 2, 0, 0.5	5 , 3, 0, 0.5	–3.5, 0.25, 0, 0.5	5, 3, 0, 0.5

Bold numbers indicate the values that differ between each run.