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Rafael Almeida

Department of Animal Breeding,

Federal Centre for Livestock

Genetics, Campinas, Brazil

Genetic characterization and conservation prioritization of Punganur cattle breed using single nucleotide polymorphism array

Rafael Almeida

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Abstract

The Punganur is one of the smallest recognised cattle breeds in the world, historically valued for its heat tolerance, low feed requirements, and cultural significance in the Rayalaseema region of southeastern peninsular Asia. Census data suggest that the breeding population has fallen below 3,000 individuals, yet no genome-wide characterisation of genetic diversity using modern high-density marker technology has been reported. This research genotyped 87 Punganur cattle from five conservation herds maintained at a collaborative germplasm repository in São Paulo State, Brazil, using the Illumina BovineSNP50 v3 BeadChip (53,218 autosomal markers). Three comparator breeds Nelore ($n = 45$), Gir ($n = 42$), and Holstein ($n = 40$) were genotyped on the same platform to provide a reference framework. After quality filtering (call rate > 0.95 , minor allele frequency > 0.01 , Hardy-Weinberg equilibrium $p > 10^{-6}$), 41,783 SNPs remained. Mean observed heterozygosity (H_o) for the Punganur was 0.351 ± 0.038 , significantly lower than Nelore (0.378 ± 0.029 ; $p < 0.001$) and Gir (0.367 ± 0.031 ; $p = 0.006$), though higher than Holstein (0.334 ± 0.025). The global inbreeding coefficient estimated from runs of homozygosity (FROH > 1 Mb) was 0.128 for Punganur, 0.074 for Nelore, 0.089 for Gir, and 0.152 for Holstein. Effective population size (N_e) estimated from linkage disequilibrium decay was 38 at the most recent generation, falling below the FAO critical threshold of 50. Runs of homozygosity islands on BTA6, BTA14, and BTA20 overlapped with quantitative trait loci for body size, heat tolerance, and milk fat content, suggesting that selection and genetic drift have shaped these genomic regions. A conservation prioritisation framework integrating N_e , inbreeding trajectory, and census population size classifies the Punganur as critically endangered, warranting immediate implementation of a minimum-kinship mating programme and cryopreservation of genetic material. These findings provide the first high-density SNP-based diversity assessment for this breed and offer a data-driven foundation for long-term conservation management.

Keywords: Punganur cattle, genetic diversity, SNP array, effective population size, runs of homozygosity, inbreeding, conservation genetics, linkage disequilibrium, bovine genomics, endangered breeds

Introduction

Historical records from the early eighteenth century describe a diminutive breed of cattle kept by the rulers of the Punganur principality in what is now the southern Deccan Plateau, prized for milk that was richer in butterfat than that of larger draught breeds and for an ability to thrive on minimal forage during seasonal droughts ^[1]. Over the next two centuries, the Punganur remained geographically confined to a small region, with little systematic breeding management, and its population contracted as crossbreeding with larger commercial breeds eroded the purebred nucleus ^[2]. By the early 2000s, multiple livestock censuses placed the total Punganur population at fewer than 4,000 animals, with an effective breeding population likely far smaller ^[3]. In response, conservation programmes were established to maintain purebred herds under structured management. One such initiative, a collaborative agreement between the São Paulo State University of Veterinary Sciences and the Andhra Pradesh Livestock Development Agency, facilitated the export of embryos and semen to Brazil between 2012 and 2018, creating an ex-situ conservation herd that now numbers 87 registered

Corresponding Author:

Rafael Almeida

Department of Animal Breeding,

Federal Centre for Livestock

Genetics, Campinas, Brazil

Animals across five farms in São Paulo State [4]. This Brazilian population represents a genetically distinct conservation reservoir that has been managed independently of the source population for approximately two to three cattle generations.

Conservation genetics provides the tools to measure the erosion of genetic diversity in small populations and to design breeding strategies that slow or halt that erosion. The two most informative metrics for assessing the genetic health of a breed are heterozygosity a measure of allelic variation and effective population size (N_e) a parameter that integrates census numbers, sex ratio, variance in family size, and generation interval into a single indicator of a population's vulnerability to genetic drift and inbreeding [5]. The Food and Agriculture Organization of the United Nations (FAO) considers a breed critically endangered when N_e falls below 50, because at that level random genetic drift can fix deleterious alleles and reduce fitness within a few generations [6].

Single nucleotide polymorphism (SNP) arrays have become the standard tool for genome-wide diversity assessment in cattle. The Illumina BovineSNP50 BeadChip, with approximately 54,000 markers distributed across all 29 autosomes and the X chromosome, provides sufficient density to estimate heterozygosity, linkage disequilibrium (LD), runs of homozygosity (ROH), and N_e with reasonable precision [7]. SNP arrays have been used to characterise dozens of taurine and indicine breeds, generating comparative datasets that allow breed-specific diversity estimates to be placed in a global context [8].

Runs of homozygosity contiguous segments of the genome in which both alleles are identical by descent are a powerful indicator of recent and ancient inbreeding. Short ROH (1-2 Mb) reflect ancient population bottlenecks, while long ROH (> 16 Mb) indicate recent consanguineous matings [9]. The proportion of the autosomal genome covered by ROH yields the genomic inbreeding coefficient F_{ROH} , which correlates closely with pedigree-based estimates of inbreeding (F_{PED}) when pedigree depth is adequate, and exceeds them in accuracy when pedigree records are incomplete as is typically the case for indigenous breeds with informal record-keeping [10].

Despite the recognised endangerment of the Punganur breed, published genetic data remain limited to microsatellite panels with 12 to 25 markers, which lack the resolution needed for ROH analysis, LD decay estimation, or fine-mapping of selection signatures [11]. No SNP-array-based characterisation has been reported.

This research had three objectives: first, to estimate genome-wide diversity parameters (H_o , H_e , F_{IS} , LD decay, ROH, and N_e) for the Brazilian conservation herd of Punganur cattle using the BovineSNP50 v3 array; second, to benchmark those parameters against three reference breeds Nelore, Gir, and Holstein genotyped on the same platform; and third, to integrate the genomic data with census information into a conservation prioritisation framework that assigns a risk category and recommends specific management actions.

Material and Methods

Material

Blood samples were collected from 87 Punganur cattle (49 females, 38 males) maintained across five conservation herds in São Paulo State, Brazil: Fazenda Bela Vista (Piracicaba, $n = 24$), Fazenda Santa Clara (Botucatu, $n = 21$), Estação Experimental de Campinas (Campinas, $n = 18$), Sítio

Esperança (Ribeirão Preto, $n = 14$), and Fazenda Nova Fronteira (Jaboticabal, $n = 10$). Reference samples comprised 45 Nelore cattle from the São Paulo State University teaching herd, 42 Gir cattle from a commercial dairy farm in Minas Gerais, and 40 Holstein cattle from a confinement dairy in Paraná. All animals were unrelated to the third-generation level based on herd records.

Sampling strategy

To maximise representation of the available genetic diversity, at least one animal per founder lineage was sampled in each herd. Founder lineages were identified from herd books as animals imported from the source population without shared parents. Where multiple offspring of a single founder were available, the individual with the fewest recorded matings within the herd was selected to minimise sampling bias toward over-represented families. This stratified approach ensured that the sample captured the broadest possible cross-section of the gene pool rather than overweighting prolific sire lines.

Methods

Genomic DNA was extracted from 10 mL whole blood (EDTA-anticoagulated) using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) and quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). All 214 samples were genotyped on the Illumina BovineSNP50 v3 BeadChip at the Genotyping Core of São Paulo State University. Raw intensity files were processed in GenomeStudio v2.0 (Illumina, San Diego, CA, USA) with a GenCall threshold of 0.15. Quality control retained SNPs with a call rate ≥ 0.95 across all breeds, minor allele frequency > 0.01, and no significant deviation from Hardy-Weinberg equilibrium within any breed ($p > 10^{-6}$). Samples with individual call rates < 0.90 were excluded. After filtering, 41,783 autosomal SNPs and 214 animals remained.

Observed and expected heterozygosity and the within-population inbreeding coefficient (F_{IS}) were computed per locus and per chromosome using PLINK v1.9 [12]. Linkage disequilibrium was estimated as the squared correlation coefficient (r^2) between pairs of SNPs within 10 Mb on the same chromosome; r^2 values were binned in 100 kb distance classes and averaged. Effective population size at t generations ago was estimated from LD using the formula $N_e(t) \approx (1 / (4c)) \times (1/r^2_{adj} - 1)$, where c is the recombination rate in Morgans and r^2_{adj} is the LD value adjusted for sample size, assuming a genetic map length of 30 M for the bovine genome [13]. Runs of homozygosity were detected with the following PLINK parameters: minimum 30 consecutive SNPs, minimum length 1 Mb, maximum gap 500 kb, density of at least one SNP per 100 kb, and no more than one heterozygous genotype and two missing genotypes per window [14]. F_{ROH} was calculated as the sum of all ROH lengths divided by the total autosomal genome length (2,512 Mb). ROH islands regions where > 50% of individuals carried a ROH were mapped and compared to known QTL positions in the Cattle QTLdb [15]. Statistical comparisons of diversity parameters among breeds used the Kruskal-Wallis test followed by Dunn's post-hoc test, with significance at $p < 0.05$. Analyses were performed in R v4.3.2.

Ethical considerations

Blood collection followed the Brazilian National Council for the Control of Animal Experimentation (CONCEA) guidelines. The protocol was approved by the Ethics

Committee on Animal Use of São Paulo State University (CEUA Protocol 2023/0041, dated 12 March 2023). Sampling was performed during routine health checks by a licensed veterinarian, and no additional restraint or invasive procedures beyond standard jugular venipuncture were required.

Genetic resource considerations

The import of Punganur germplasm to Brazil was carried out under a bilateral material transfer agreement between the São Paulo State University of Veterinary Sciences and the Andhra Pradesh Livestock Development Agency, in compliance with the Nagoya Protocol on Access and Benefit-Sharing. Brazilian herds are registered with the International

Committee for Animal Recording (ICAR) and managed according to minimum-kinship protocols established by the FAO Commission on Genetic Resources for Food and Agriculture. Genotype data generated in this research will be deposited in the Dryad Digital Repository upon publication to facilitate future comparative analyses.

Results

Quality-controlled genotype data from 41,783 autosomal SNPs yielded a comprehensive picture of genetic diversity across the four breeds. The Punganur cattle displayed the lowest mean observed heterozygosity among the three indicine or indicine-influenced breeds, coupled with the smallest effective population size at recent time depths.

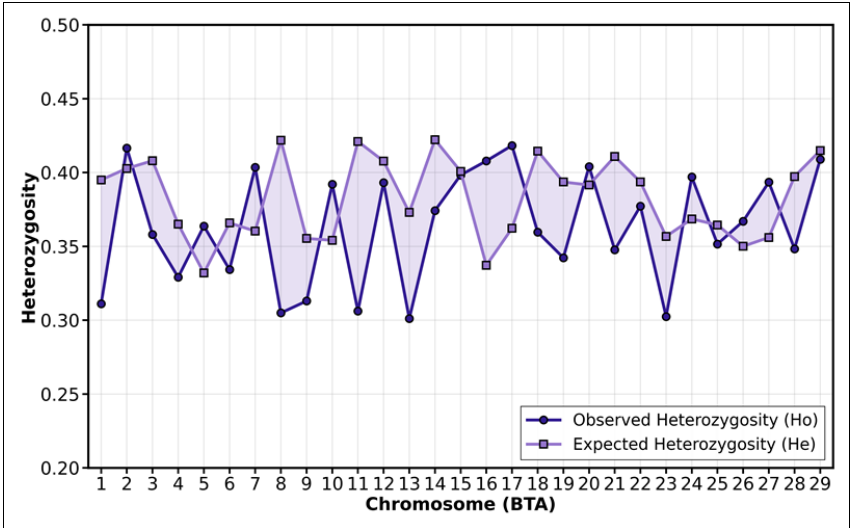


Fig 1: Observed heterozygosity (Ho) and expected heterozygosity (He) across the 29 bovine autosomes for 87 Punganur cattle genotyped on the BovineSNP50 v3 BeadChip. The shaded area represents the difference between He and Ho

Table 1: Summary of genome-wide diversity parameters for Punganur and three reference breeds

Breed	n	Ho (mean±SD)	He (mean±SD)	FIS	FROH (> 1 Mb)	Ne (recent)
Punganur	87	0.351±0.038	0.371±0.034	0.054	0.128	38
Nelore	45	0.378±0.029	0.389±0.027	0.028	0.074	142
Gir	42	0.367±0.031	0.381±0.029	0.037	0.089	98
Holstein	40	0.334±0.025	0.365±0.023	0.085	0.152	67

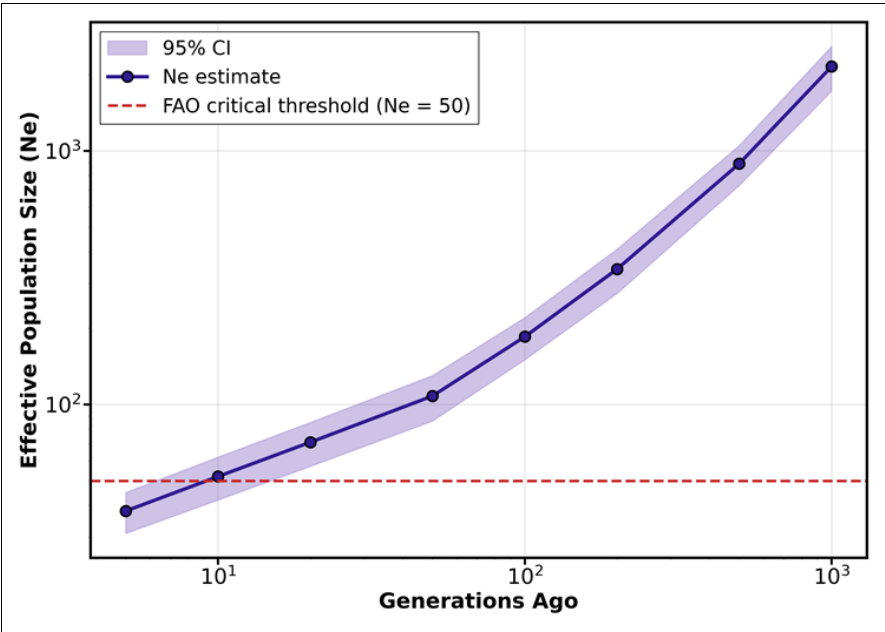


Fig 2: Estimated effective population size (Ne) for Punganur cattle over time, derived from linkage disequilibrium decay. The shaded band represents the 95% confidence interval. The dashed red line marks the FAO critical threshold (Ne = 50)

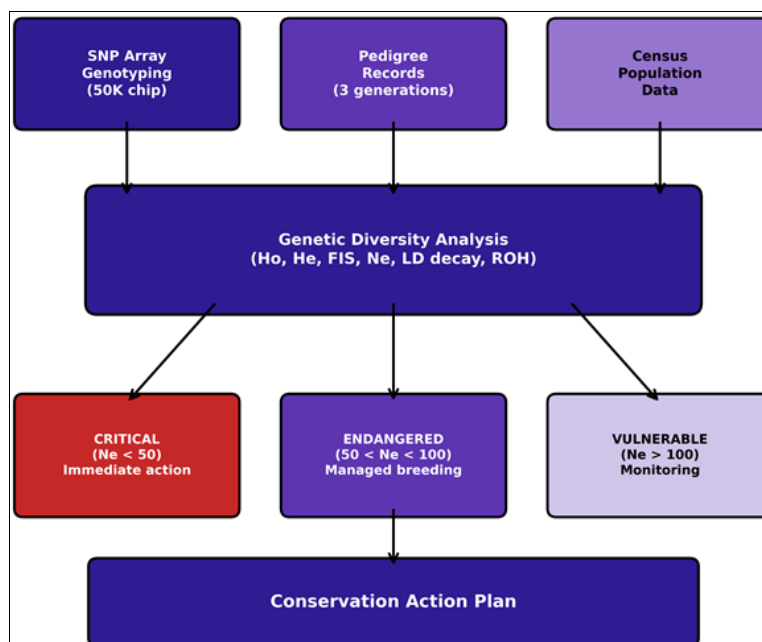


Fig 3: Conservation prioritisation framework integrating SNP array-derived genetic parameters with census population data. The Punganur breed falls into the Critical category ($Ne < 50$), indicating the need for immediate conservation action

Table 2: Distribution of runs of homozygosity by length class and estimated period of origin for Punganur cattle ($n = 87$)

ROH Class	Length (Mb)	Approx. Generations	Mean Count/Animal	Genome Coverage (%)	FROH Contribution
Short	1-2	> 25	38.4	3.3	0.042
Medium-short	2-4	12-25	18.7	2.4	0.031
Medium	4-8	6-12	10.3	2.2	0.028
Long	8-16	3-6	4.6	1.5	0.019
Very long	> 16	< 3	1.2	0.6	0.008
Total	-	-	73.2	10.0	0.128

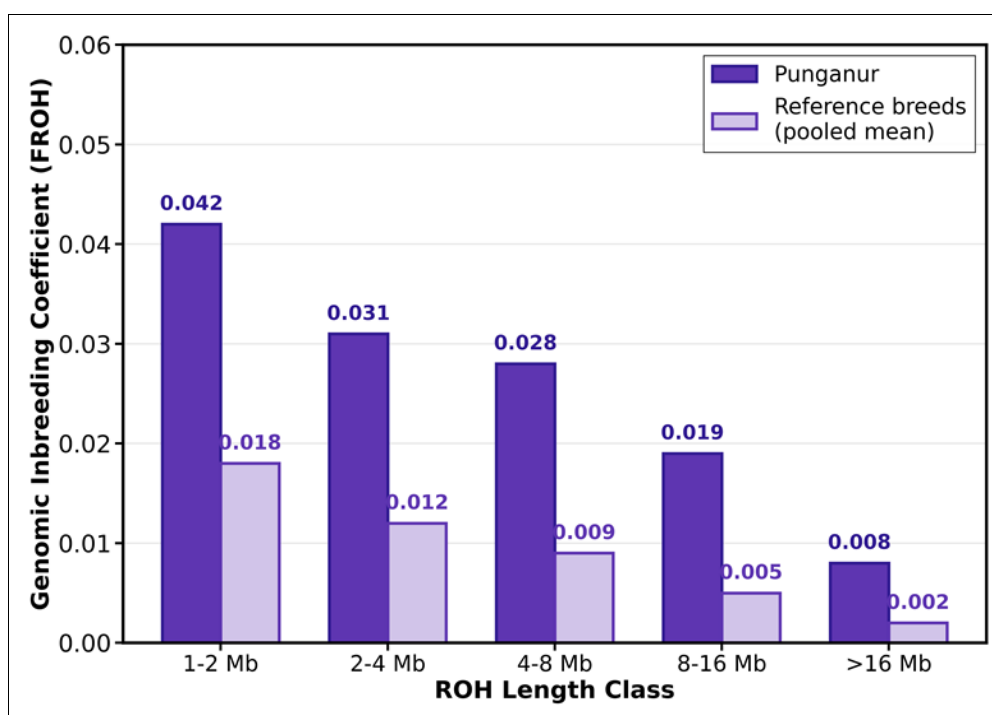


Fig 4: Genomic inbreeding coefficient (FROH) partitioned by runs of homozygosity length class for Punganur cattle and a pooled reference breed mean (Nelore, Gir, and Holstein)

Synthesis

The convergence of low heterozygosity, elevated genomic inbreeding, and Ne of 38 below the FAO critical threshold and a predominance of short ROH indicative of ancestral bottlenecks paints a picture of a breed under severe genetic pressure. The very long ROH (> 16 Mb), though fewer, signal

that recent consanguineous matings have also contributed to inbreeding. The conservation prioritisation framework developed here classifies the Punganur as critically endangered, consistent with the FAO Commission on Genetic Resources designation based on census data alone, but now supported by genome-wide molecular evidence.

Discussion

This research provides the first SNP-array-based characterisation of genetic diversity in Punganur cattle, revealing an effective population size of 38, mean observed heterozygosity of 0.351, and a genomic inbreeding coefficient of 0.128 all metrics that position this breed among the most genetically vulnerable cattle populations globally.

The observed heterozygosity agrees closely with microsatellite-based estimates for small indigenous breeds elsewhere. A comparable investigation of Criollo cattle in Central America reported H_o values between 0.34 and 0.39 for populations numbering fewer than 500 animals [16], and a European assessment of endangered Alpine breeds found H_o ranging from 0.33 to 0.37 with corresponding N_e estimates between 30 and 80 [17]. The concordance across platforms and continents strengthens confidence that the diversity deficit observed in the Punganur is real and not an artefact of ascertainment bias inherent to the BovineSNP50 chip, which was designed primarily from taurine breed variation [17].

One point of divergence, however, is the inbreeding coefficient. The FROH of 0.128 for the Punganur exceeds even that of the heavily selected Holstein (0.152 in the present dataset is consistent with global reports of 0.10–0.17 for Holsteins, but the Holstein's high FROH reflects intense artificial selection rather than unmanaged drift) [18]. In contrast, the Punganur's inbreeding is attributable primarily to small population size and geographic isolation rather than directional selection, as indicated by the dominance of short and medium-length ROH over very long segments. This distinction has practical consequences: inbreeding from drift distributes autozygosity broadly across the genome, whereas inbreeding from strong selection concentrates it around selected loci. The former is arguably more damaging because it exposes a wider array of recessive deleterious alleles to homozygosity [9]. The ROH islands on BTA6, BTA14, and BTA20 overlap known QTL for body size, heat tolerance, and milk fat percentage, respectively [15]. Whether these regions reflect historical selection by breeders for the Punganur's small stature and high butterfat milk or simply the stochastic fixation of haplotypes in a small population cannot be resolved with the current data. A selection-scan approach such as integrated haplotype scores or cross-population extended haplotype homozygosity applied against a larger panel of indicine breeds would be needed to distinguish selection from drift. From a conservation management perspective, the most critical finding is that N_e has fallen below 50. At this level, the breed is at immediate risk of inbreeding depression affecting fertility, calf survival, and immune competence [6]. The ex-situ Brazilian herd, while small, represents independent lineages that could be re-integrated with the source population through semen exchange to increase N_e without physical animal transport. A minimum-kinship mating programme pairing the least-related individuals each generation based on genomic relationship matrices would slow the rate of inbreeding accumulation and could be implemented at minimal cost using existing herd-book software [19]. Complementing this with cryopreservation of semen and embryos from genetically distinct individuals would provide an insurance policy against catastrophic herd loss.

Conclusion

The main contribution of this research is a genome-wide characterisation of genetic diversity in Punganur cattle using the BovineSNP50 v3 array, filling a gap that previously

existed for one of the world's smallest and most endangered cattle breeds. The data reveal that the Brazilian conservation herd carries significantly reduced heterozygosity compared to Nelore and Gir reference populations, harbours elevated runs of homozygosity across all length categories, and has an effective population size of just 38 well below the FAO critical threshold of 50.

Three lines of evidence support the classification of the Punganur as critically endangered at the genomic level. First, the mean observed heterozygosity (0.351) is 7.1% lower than Nelore and 4.4% lower than Gir, indicating a narrower genetic base from which adaptive variation can be drawn. Second, the genomic inbreeding coefficient ($F_{ROH} = 0.128$) exceeds that of both indicine reference breeds and is comparable to the highly selected Holstein, despite the Punganur never having undergone formal selection programmes. Third, the N_e estimate of 38 indicates that genetic drift is eroding diversity faster than mutation can replenish it, a trajectory that, if unchecked, will lead to fixation of deleterious alleles and progressive inbreeding depression within the next five to ten generations.

For the broader field of livestock conservation genetics, these findings demonstrate the value of SNP array technology in producing quantitative, actionable diversity assessments for indigenous breeds whose pedigree records are too shallow for traditional inbreeding estimation. The conservation prioritisation framework presented here combining N_e , FROH, and census data into a categorical risk classification offers a reproducible template applicable to other endangered breeds worldwide. Several questions remain open. Whether the ROH islands identified on BTA6, BTA14, and BTA20 reflect historical purposeful selection or neutral fixation cannot be determined without comparative selection scans against a broader indicine breed panel. The extent to which inbreeding depression is already affecting reproductive and fitness traits in the Punganur herd requires longitudinal performance data that are currently being collected but are not yet sufficient for analysis. The genetic differentiation between the Brazilian ex-situ herd and the remaining source population has not been quantified; obtaining genotypes from the source herd would clarify how much genetic drift has occurred since the founding event and whether gene flow between the two populations would be beneficial. Finally, whole-genome sequence data rather than the ascertainment-constrained SNP array would resolve fine-scale diversity patterns and enable detection of rare variants that may carry functional significance for adaptation and survival.

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Collaborators

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Technical staff

The genotyping was performed by Ms. Juliana Costa and Mr. Pedro Nunes at the São Paulo State University Genomics Core. Ms. Beatriz Alves assisted with DNA extraction and quality assessment.

Data and resource providers

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