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PNAS RESEARCH ARTICLE | EVOLUTION

The importance of small-island populations for the long-term survival of endangered large-bodied insular mammals

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Island populations of large vertebrates have experienced higher extinction rates than mainland populations over long timescales due to demographic stochasticity, genetic drift, and inbreeding. While being more susceptible to extinction and as such potentially targeted for conservation interventions such as genetic rescue, small-island populations can experience relatively less anthropogenic habitat degradation than those on larger islands. Here, we determine the consequences and conservation implications of long-term isolation and recent human activities on genetic diversity of island populations of two forest-dependent mammals endemic to the Wallacea archipelago: the anoa (*Bubalus* spp.) and babirusa (*Babyrousa* spp.). Using genomic analyses and habitat suitability models, we show that, compared to closely related species, populations on mainland Sulawesi exhibit low heterozygosity, high inbreeding, a high proportion of deleterious alleles, and experience a high rate of anthropogenic disturbance. In contrast, populations on smaller islands occupy higher-quality habitats, possess fewer deleterious mutations despite exhibiting lower heterozygosity and higher inbreeding. Site frequency spectra indicate that these patterns reflect stronger, long-term purging in smaller-island populations. Our results thus suggest that conservation efforts should focus on protecting small-island high-quality habitats and avoiding translocations from mainland populations. This study highlights the crucial role of small offshore islands for the long-term survival of Wallacea's iconic and indigenous mammals in the face of development on the mainland.

conservation | genetic rescue | genetic load | inbreeding | habitat suitability modeling

Significance

Within tropical archipelagos, such as the Wallacea biodiversity hotspot, larger islands experience greater resource exploitation compared to smaller ones, highlighting the potential of smaller islands as refuges for conservation. To investigate the genetic health of populations on small islands, we used genomic, occurrence, and environmental data from a system of replicated populations of anoa and babirusa across islands of varying sizes. In contrast to larger islands like Sulawesi, our results demonstrate that smaller offshore islands not only provide higher-quality habitats but also support populations that have efficiently purged harmful mutations. Thus, despite their known vulnerability over geological time-frames, small-island populations can provide long-term insurance against human-driven extinction and conservation efforts should prioritize habitat management over translocations.

Throughout the Quaternary period, island populations of large vertebrates experienced higher rates of extinction than their mainland counterparts (1). This pattern can be attributed to demographic stochasticity, as well as the strong effects of genetic drift that operate in small populations of slow-reproducing species, and the associated negative consequences for fitness and adaptability (2, 3).

In the past century, these long-term threats to island populations have been further compounded by human activities such as urbanization, the introduction of invasive species, land conversion for agriculture, mining, and hunting (4). These anthropogenic pressures often vary across islands, with smaller and more isolated populations typically facing less intense exploitation compared to larger, more accessible ones (5, 6). Consequently, while populations on small islands may be more prone to extinction over geological timescales, they can be less affected by habitat degradation.

Genetic rescue, typically involving the translocation of individuals from larger to smaller populations, can be an effective strategy for increasing diversity in genetically depauperate populations (7, 8). Translocating unrelated individuals introduces novel alleles, which can mask the expression of deleterious recessive alleles and alleviate inbreeding depression in the recipient population. In addition, a small subset of these alleles may enhance the recipient population's potential to adapt to changing environments, provided that newly introduced variation confers a substantial selective advantage, and the effective population size is sufficiently large for selection to operate and counteract the effects of genetic drift (9).

Although genetic rescue has yielded notable successes, a recent empirical study (10) and simulations (11) suggest that it can also lead to the introduction of harmful alleles in small populations. This can negatively impact the survival of historically small populations, which have undergone long-term purging and as a result often carry an overall lower number of deleterious alleles than large populations (12). In fact, simulations suggest that historically small populations are likely to face a lower risk of short-term

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extinction following a bottleneck compared to larger populations (attributable to lower number of harmful alleles), but a higher risk of long-term extinction due to their limited adaptive potential (attributable to reduced genetic diversity) in the face of environmental change (12, 13). This highlights the need for careful genetic management, especially in historically small populations in which natural selection has likely eliminated many harmful alleles, but which still face fitness challenges due to inbreeding and lower adaptive potential due to low genetic diversity (12–14).

The archipelago of Wallacea in Indonesia provides a natural laboratory to investigate the combined effects of long-term isolation and recent human activity on island population genetic structure and viability. Due to its long geological history of isolation, Wallacea is a global hotspot for endemism. In recent years, it has emerged as a frontier region for development in Indonesia, and its ecosystems and unique species face increasing pressures from deforestation and mineral extraction (5, 6, 15). To date, human activities have been concentrated in the more accessible lowland areas on Sulawesi, whereas some smaller offshore islands have remained less disturbed (5).

Here, we determine the consequences and conservation implications of long-term isolation and recent human activities on the genomic diversity of island populations of two forest-dependent mammals, the anoa *Bubalus* spp. and babirusa (*Babyrousa* spp.) (Fig. 1 *A* and *B*, respectively). Both taxa are endemic to Wallacea and are broadly codistributed on islands of different sizes across the archipelago. Previous work suggests they underwent range expansions approximately 2 Mya (16), however, current populations are highly fragmented following several decades of population declines and local extinctions due to habitat loss and hunting.

As endangered and endemic mammals, the anoa and babirusa are the focus of ex situ breeding programs and viewed as potential candidates for conservation programs aimed at increasing the size and genetic diversity of wild populations. Here, we use a combination of population genomic analyses and habitat suitability models, to evaluate the persistence of populations on islands of different sizes and in varying habitat quality to investigate the stability of small populations. Our findings underscore the critical role of small offshore islands in ensuring the long-term survival of Wallacea's iconic mammals amid ongoing land-use changes on the Sulawesi mainland.

Results and Discussion

We generated short-read sequence data from samples of anoa (n = 67) and babirusa (n = 46) collected from across their respective ranges in Wallacea. To determine broad population genetic structure, we mapped short reads of each taxon to a reference genome of a conspecific, or, in the case of the anoa, the water buffalo (*Bubalus bubalis*) to maximize coverage (*SI Appendix*, Fig. S1 and Dataset S1). We performed principal components (Fig. 1 *C* and *D*), phylogenetic, and admixture analyses (*SI Appendix*, Fig. S2) with 1,053,534 unlinked SNPs for anoa, and 1,011,533 unlinked SNPs for babirusa (thinned for computational efficiency, see *SI Appendix*). These analyses revealed concordant patterns of population structure across both taxa (Fig. 1 *C* and *D*), in which three distinct lineages were identified corresponding to individuals from North Sulawesi, Southeast Sulawesi, and the small offshore islands of Buton (anoa) or Togean (babirusa). This population structure, which broadly supports previous findings based on mitochondrial and microsatellite data (16), was then used to define populations in subsequent analyses.

Populations on Smaller Islands Are Less Genetically Diverse than on Mainland Sulawesi. To determine the consequences of long-term isolation, we quantified levels of genetic diversity in populations on Sulawesi and smaller nearby islands (Buton and Togean). To do so, we computed genome-wide Watterson's θ, an unbiased estimator of heterozygosity in a single diploid individual under the infinite sites model (18), using ROHan (19). We found that θ on the small islands of Togean (babirusa: mean = 0.29×10^{-3}, SE = 0.02×10^{-3}) and Buton (anoa: mean = 0.58×10^{-3}, SE = 0.02×10^{-3}) was ~twofold to ~sixfold lower than in populations from mainland Sulawesi (babirusa: mean = 1.85×10^{-3}, SE = 0.1×10^{-3}; anoa: mean = 1.33×10^{-3}, SE = 0.1×10^{-3}), indicating that small-island populations possess less genetic diversity than populations on the larger island of Sulawesi (Fig. 2*A*). On Sulawesi, however, we found that babirusa from the Southeast Sulawesi possessed lower θ (mean = 1.17×10^{-3}, SE = 0.04×10^{-3}) compared to those found on the Northern peninsula (mean = 2.17×10^{-3}, SE = 0.06×10^{-3}).

We then tested whether anoa and babirusa populations are characterized by more variable levels of genetic diversity compared to related species that do not occur on islands of varying size. To address this, we compared levels of genetic variability (Watterson's θ) and inbreeding (ROHs greater than 1 Mbp) among anoa and babirusa populations to those observed in 19 closely related taxa (52 individuals in total), including highly endangered species such as the pygmy hog (*Porcula salvianus*), the Javan warty pig (*Sus verrucosus*), and banteng (*Bos javanicus*). Levels of genetic diversity (Watterson's θ) were on average lower in anoa than in other bovid species (Fig. 2*A*). The same was observed in babirusa, which aside from the North Sulawesi population, possessed lower levels of genetic diversity than most other suids. Lower levels of genetic diversity in the Wallacean endemics is likely the result of smaller effective population sizes resulting from their island habitats and/or recent bottleneck(s).

To assess whether populations showed signs of recent bottlenecks, we analyzed runs of homozygosity (ROHs) using ROHan (19) and reconstructed demographic histories using GONE (20) (*SI Appendix*). Consistent with genome-wide patterns of genetic diversity, ROH were shorter and less abundant in Northern Sulawesi babirusa and in Northern and Southeastern Sulawesi anoa (Fig. 2*B* and *SI Appendix*, Fig. S3), indicative of lower levels of inbreeding compared to other populations. Demographic reconstructions based on linkage disequilibrium (*SI Appendix*, Fig. S4) supported these findings indicating that Northern Sulawesi babirusa (Ne ~ 10,000) and anoa from both the North (Ne ~ 20,000) and Southeast (Ne ~ 5,000) regions of Sulawesi have maintained substantial population sizes, remaining stable for at least 200 generations.

Small populations from the small offshore islands of Buton and Togean showed different demographic histories than populations from the larger island of Sulawesi for both anoa and babirusa. Anoa from Buton possessed longer ROHs than those from mainland Sulawesi (Fig. 2*C* and *SI Appendix*, Fig. S3), and demographic reconstruction indicated that they formed part of a stable, yet substantially smaller population (Ne ~200 to 400) than mainland anoa (*SI Appendix*, Fig. S4). Similarly, babirusa from the island of Togean possessed longer ROHs than those from Northern Sulawesi (Fig. 2*C*). Demographic reconstruction suggested that Togean babirusa underwent a strong population bottleneck ~50 to 100 generations ago (*SI Appendix*, Fig. S4). The inferred magnitude and the timing of this bottleneck, however, were strongly affected by the choice of parameters used for the GONE analysis (*SI Appendix*, Fig. S4) and the results are inconsistent with the fact that, as for Buton anoa, they possess similar

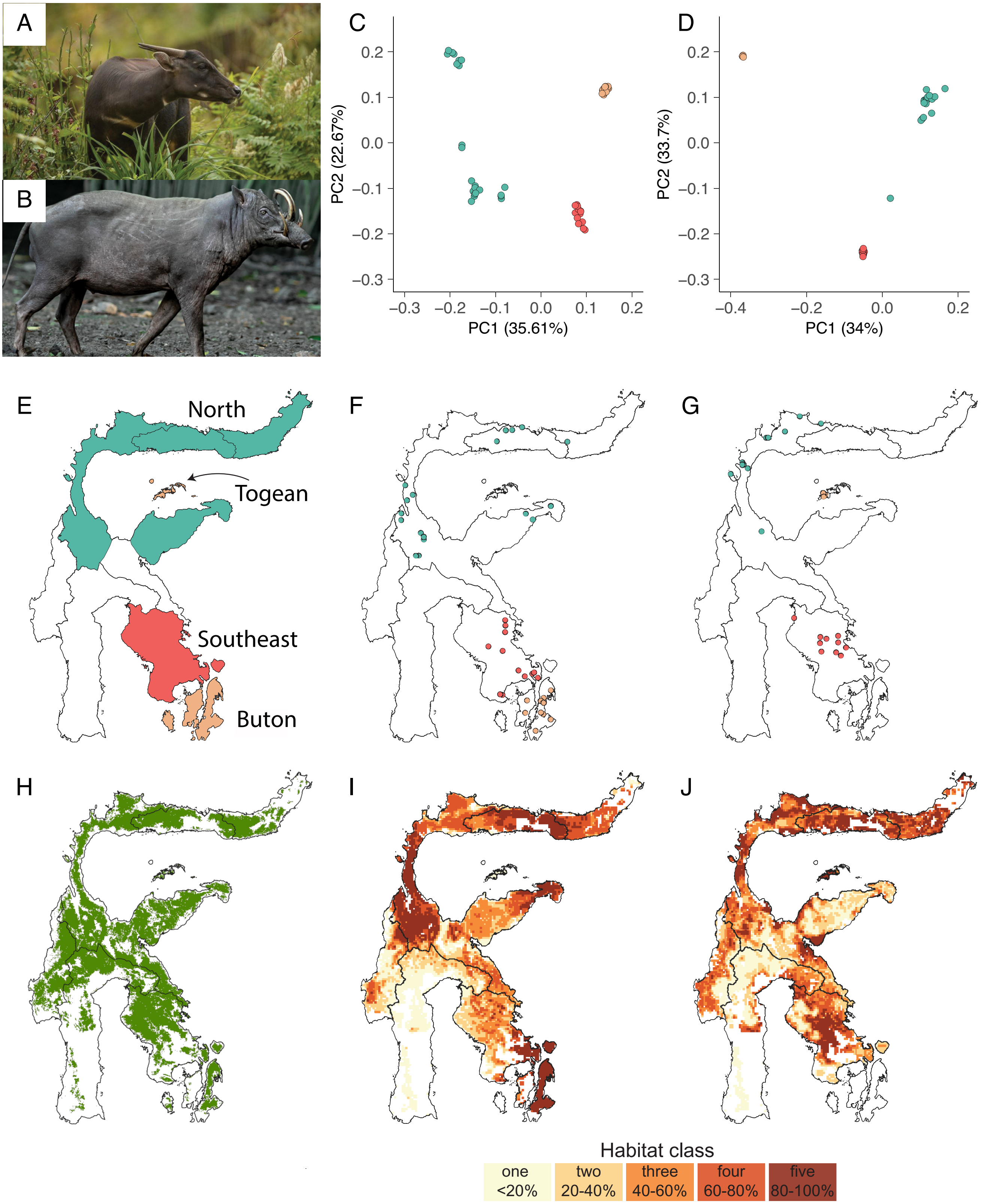

Fig. 1. Population structure and habitat suitability. (*A*) Photographs of anoa (*Top*, © Chester Zoo) and (*B*) babirusa (*Bottom*, photo credit: S.L. Mitchell). (*C*) Principal component analysis based on SNPs from genomes (>5×) for anoa (*Middle*, 53 individuals; 1,053,534 SNPs) and (*D*) babirusa (*Right*, 37 individuals; 1,011,533 SNPs), (*E*) Zones of endemism (*Right*) based on Frantz et al. (16). (*F*) Locations of samples for anoa (*Left*), (*G*) babirusa (*Right*) colored based on population structure (*SI Appendix*, Fig. S2), (*H*) Sulawesi and offshore islands, showing the 2018 forest cover [Global Forest Change repository, v1.6, Hansen et al. (17), processed in Voigt et al. (5)] in green used to constrain the ensemble distribution models of habitat suitability for (*I*) anoa (*Middle*) and (*J*) babirusa (*Right*) categorized by the habitat suitability score quantile (class one = least suitable habitat, class five = most suitable habitat.

levels of genetic diversity within and outside ROHs (Fig. 2*A*), which is indicative of a stable population. Combined with this evidence, we suggest that the population bottleneck identified by GONE could be an artifact of the low sample size of the Togean babirusa genome with over 5× depth of coverage (n = 5) compared to Buton anoa (n = 17).

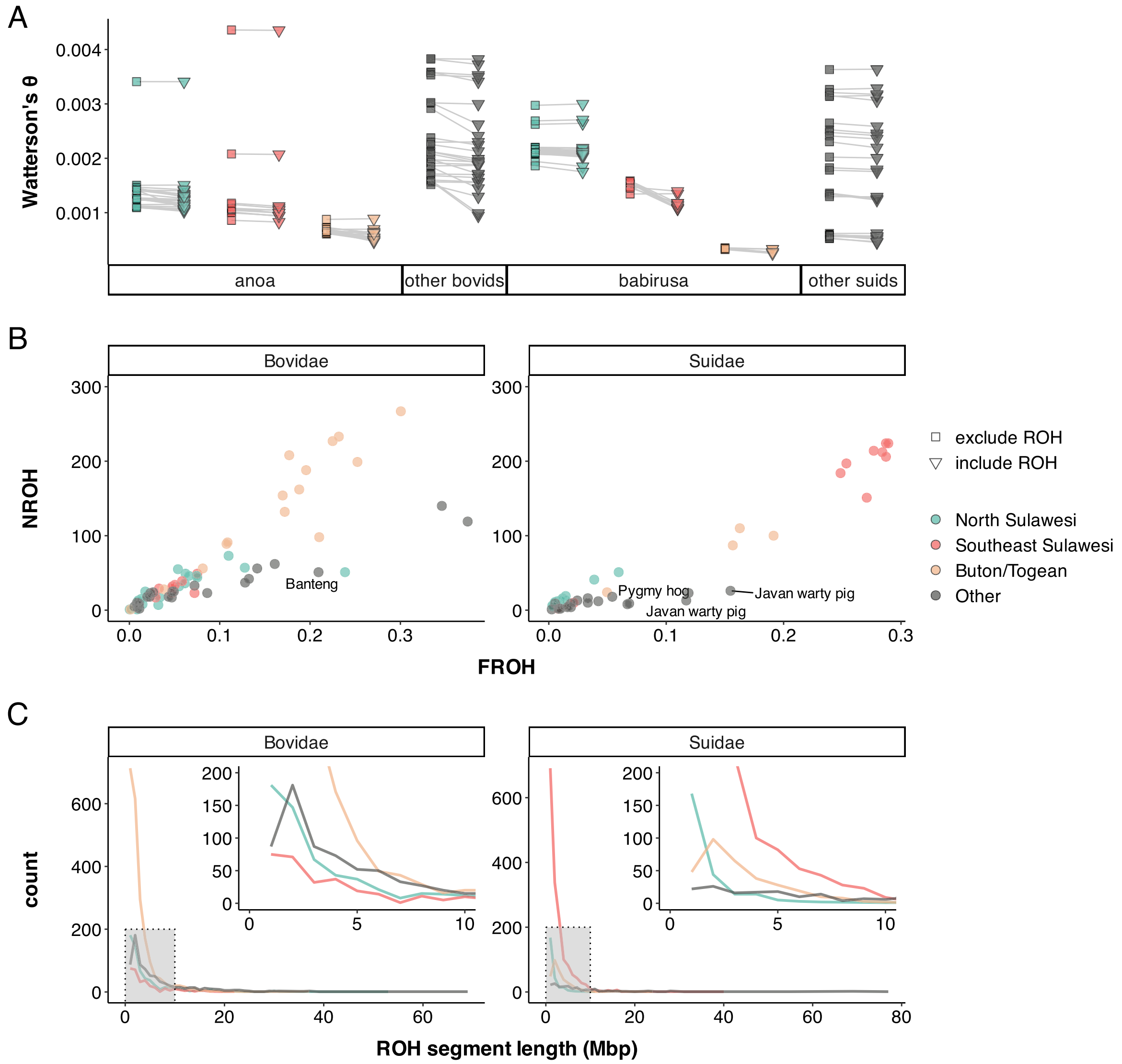

Fig. 2. Population-level genetic diversity and ROHs. (*A*) Genome-wide Watterson's θ computed across the whole genome (triangle) and excluding ROH (square) in anoa, babirusa, and their respective close relatives, i.e., Bovidae for anoa and Suidae for babirusa. (*B*) Sum of size of ROH segments across the genome (NROH) and proportion of ROH across the genome (FROH) (*C*) distribution of ROH segment length.

Southeast Sulawesi babirusa possessed more ROHs and had a larger portion of their genome in ROH than any other population of babirusa and any other suid species (Fig. 2*B*). Interestingly, despite this high level of inbreeding, Southeast Sulawesi babirusa possessed average levels of genetic diversity when compared to other suid species (Fig. 2*A*). Their level of genetic diversity, however, was significantly higher when excluding ROHs ($P < 0.001$; Fig. 2*B*). The significant change in genetic diversity when excluding ROHs suggests that babirusa living in Southeast Sulawesi were part of a historically large population that underwent a recent bottleneck. Demographic reconstruction, based on 11 of the genomes with depth of coverage greater than 5×, indicated that Southeastern babirusa formed part of a small population (Ne ~ 200 to 400) which underwent a 5 to 10-fold population bottleneck ~100 to 150 generations ago (*SI Appendix*, Fig. S4).

Impact of Anthropogenic Disturbances on Demography. To address whether the dramatic bottleneck in Southeast Sulawesi babirusa is consistent with a recent decrease of suitable habitat in the area, we constructed an ensemble species distribution model (21) based on bioclimatic variables and forest cover (5, 17) (*SI Appendix*, Table S1). Our models reveal a substantial difference in habitat suitability on mainland Sulawesi, with the northern region displaying approximately 3.8 times more suitable habitat for anoa and 1.4 times more for babirusa than the Southeast Peninsula (*SI Appendix*, Table S2). This trend is consistent with recent deforestation rates (between 2000 and 2017), which were higher in Southeast Sulawesi compared to the North (22), suggesting that deforestation has led to the reduced habitat availability detected by our models. These data, combined with previously reported high rates of poaching (23), suggest that the recent population bottleneck in babirusa in Southeast Sulawesi has been driven by relatively recent anthropogenic disturbances, rather than longer-term evolutionary processes.

Our data, however, indicate that the Southeastern anoa population did not experience a bottleneck, suggesting that anthropogenic disturbances had a lesser impact on this species compared to babirusa. Spatial analyses revealed that deforestation is more prevalent in lowland areas, evidenced by the substantially higher mean altitude of forested areas (505 m) in the Southeastern peninsula relative to deforested areas (95 m). This altitudinal difference may account for the observed discrepancy between anoa and babirusa, potentially reflecting a greater tolerance of anoa for higher-altitude forest habitats.

In contrast to the mainland, smaller offshore island of Togean (babirusa) and Buton (anoa) possessed proportionally more suitable habitat (i.e., top 20% of all suitable habitat; see *SI Appendix, Supplementary Text*; 1.5-fold more highly suitable area for anoa and ~1.6 for babirusa relative to North Sulawesi; Fig. 1*D* and *SI Appendix*, Table S2). In addition, Buton and Togean have a higher percentage of land (14% and 45% respectively) within protected areas (IUCN category I-V), compared to North (14%) and Southeast (6%) Sulawesi. When relating the distribution of suitable habitat to different land use classes, we found, for both species, a considerable overlap with areas that have protected status (*SI Appendix*, Fig. S5). This highlights the effectiveness of the protected area network for conserving habitat for Wallacea's endemic mammals. Combined with our genomic results, this indicates that Buton anoa, also potentially Togean babirusa, form part of a historically small, yet stable population that inhabits a highly suitable and already protected habitat (Fig. 1*D* and *SI Appendix*, Fig. S5 and Table S2).

More Deleterious Alleles Segregate on Sulawesi than on Small Islands. Recent studies of several large-bodied mammals indicate that small populations with long histories of isolation [e.g., Channel Island foxes (24), Iberian lynx (25), Bengal tigers (26), and mammoths (27)] show evidence of accumulation of mildly deleterious alleles, while naturally purging strongly deleterious recessive variants. To evaluate how small-island populations of anoa and babirusa purge deleterious alleles compared to their mainland populations, we computed genetic load in individual genomes, using three conservation scores: SIFT, PhyloP, and phastCons (*SI Appendix*). To allow cross-species comparisons, we estimated genetic load for loci that show one-to-one orthology between the pig and cow genomes (*SI Appendix*). We first computed a load score by summing conservation scores, weighted by genotype probability for derived alleles found at homozygous states across the genome (*SI Appendix, Supplementary Methods*). This homozygous load represents the minimum impact of deleterious alleles on fitness (i.e., assumes that all deleterious alleles are recessive). We also computed the heterozygous load by summing the scores of derived alleles found at heterozygous states and the total load by summing the impact of alleles found at both homozygous and heterozygous states (total load) across the genome. The total load represents the maximum impact of deleterious alleles on fitness (i.e., assumes that all deleterious alleles are dominant).

Total, heterozygous, and homozygous load were significantly lower in anoa than in babirusa ($P < 0.001$; see *SI Appendix*) (Fig. 3 *A* and *B* and *SI Appendix*, Fig. S6 and Table S3). Mean total load, and heterozygous load, calculated for all individuals from the same population, were also significantly higher in large populations found on mainland Sulawesi than in smaller island populations such as Togean (babirusa) and Buton (anoa; Fig. 3*C* and *SI Appendix*, Fig. S6 and Table S3). This result indicates that deleterious alleles, found at heterozygous states, are more abundant in larger, mainland, populations than in smaller, island populations.

Mean homozygous load, however, was significantly higher in small-island populations than in larger island populations (Fig. 3*D*). Altogether, these results indicate that large populations possess more deleterious recessive alleles overall, most of which are found in heterozygous state and therefore hidden from selection. Thus, although large populations possess more deleterious alleles, the overall fitness impact of deleterious alleles is likely to be higher in smaller island populations which possess more deleterious alleles in homozygous state.

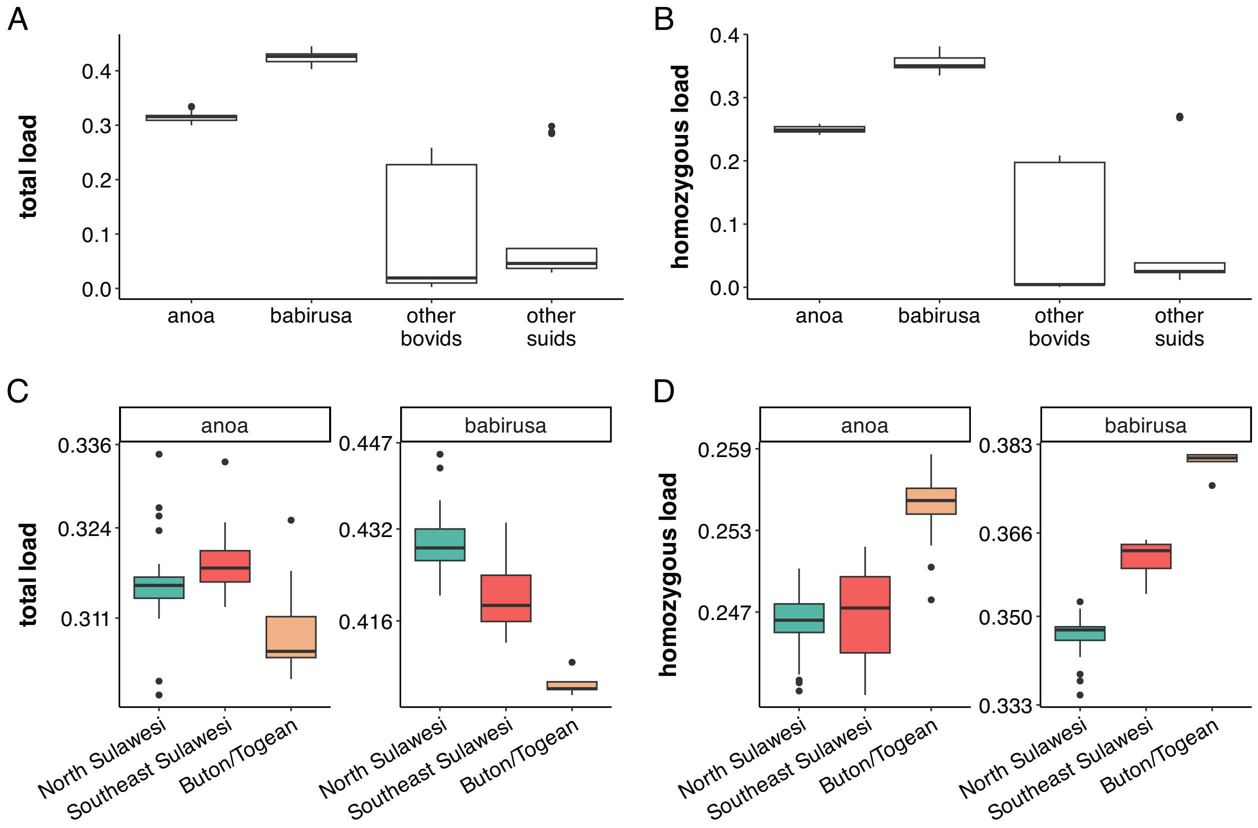

Fig. 3. Genetic load in babirusa, anoa, and closely related species. (*A*) Total load computed in individual genomes using SIFT scores for alleles found at both heterozygous and homozygous states in anoa, babirusa, and their close relatives. (*B*) Homozygous load computed in individual genomes using SIFT scores for alleles found at homozygous states in anoa, babirusa, and their close relatives. (*C*) Interisland comparison of total load and (*D*) homozygous load within the populations of anoa and babirusa.

Small-Island Populations Efficiently Purge Deleterious Alleles. The difference between the total and homozygous load observed in small and large populations could be due to either lower levels of heterozygosity or the lower efficiency of purifying selection in small populations (Fig. 2*B*). To assess the efficiency of purifying selection across different populations, we first built unfolded site frequency spectra (SFS) using ANGSD (28), based on alleles that have been assigned different impact ratings by the Variant Effect Predictor (29), i.e., low, modifier, moderate, and high (Fig. 4 and *SI Appendix*). Comparing SFS provides the opportunity to assess the impact of purifying selection on the full frequency range of deleterious alleles in a population, not just those in heterozygous and homozygous states.

We found that SFS showed an excess of low-frequency deleterious alleles in mainland populations that have not experienced bottleneck(s), such as populations of anoa in Southeast Sulawesi (Fig. 4*B*) and of both taxa in North Sulawesi (Fig. 4 *A* and *D*). This pattern is consistent with the effect of purging reducing the frequency of deleterious alleles (24–27). The persistence of deleterious alleles at low frequency in these large, highly heterozygous, populations is likely due to their recessive nature, which means they are less likely to be exposed to selection than in smaller, less heterozygous, populations.

In contrast, we found fewer deleterious alleles at low frequency in the recently bottlenecked Southeast Sulawesi babirusa, compared to in the North Sulawesi population. This is likely due to a weaker effect of purging and stronger drift in Southeast Sulawesi babirusa than in the North Sulawesi. Both small-island populations, however, possessed many fixed deleterious alleles, but had fewer deleterious alleles at low frequency than in large populations and in the recently bottlenecked Southeast babirusa population. The high degree of fixed deleterious alleles is likely to be the result of strong genetic drift in small populations. Reduced heterozygosity in these island populations likely explains the near absence of low-frequency deleterious alleles, a result of long-term purging where recessive alleles are exposed to selection. This is in contrast with large populations, in which deleterious alleles persist at low frequency despite purging.

To quantitatively compare the shape of the SFS across four impact ratings, we used a composite likelihood (CL) approach adapted from Nielsen et al. (30). Large differences in CL values across impact ratings point to contrasting shapes of the SFS. As the low-impact alleles are less likely to be affected by selection compared to the other three impact ratings (modifier, moderate, high), we used the SNPs in this rating as the expected SFS. For each impact rating, we obtained 1,000 CL values across bootstrap replicates (*SI Appendix*, *Supplementary Methods*), which were normalized using the mean of the bootstrap value of the low impact CL. In large island populations, normalized CL distributions (across bootstrap replicates) of the three higher impact ratings were centered around one (Fig. 4 *A*, *B*, *D*, and *E*). This indicates that the SFS of the higher impact ratings were not quantitatively different from that of the SFS built using low-impact ratings. In contrast, in small-island populations, the mean CL across the three higher impact ratings was higher than one, indicating that the shape of the SFS of the higher impact alleles is quantitatively different from the shape of the SFS built using low impact alleles (Fig. 4 *C* and *D*).

The observed differences in the shape of the SFS across different impact ratings in the small-island populations suggest that, although their population size is smaller than their mainland counterparts, purging is having a stronger distorting effect on the SFS of more impactful alleles. This is likely the result of recessive deleterious alleles that are more likely to be exposed to selection in a less heterozygous population. Over time, this will lead to a reduction in frequency for the most deleterious alleles in small populations, consistent with our total load scores (Fig. 3*A*) and a previous simulation study (11).

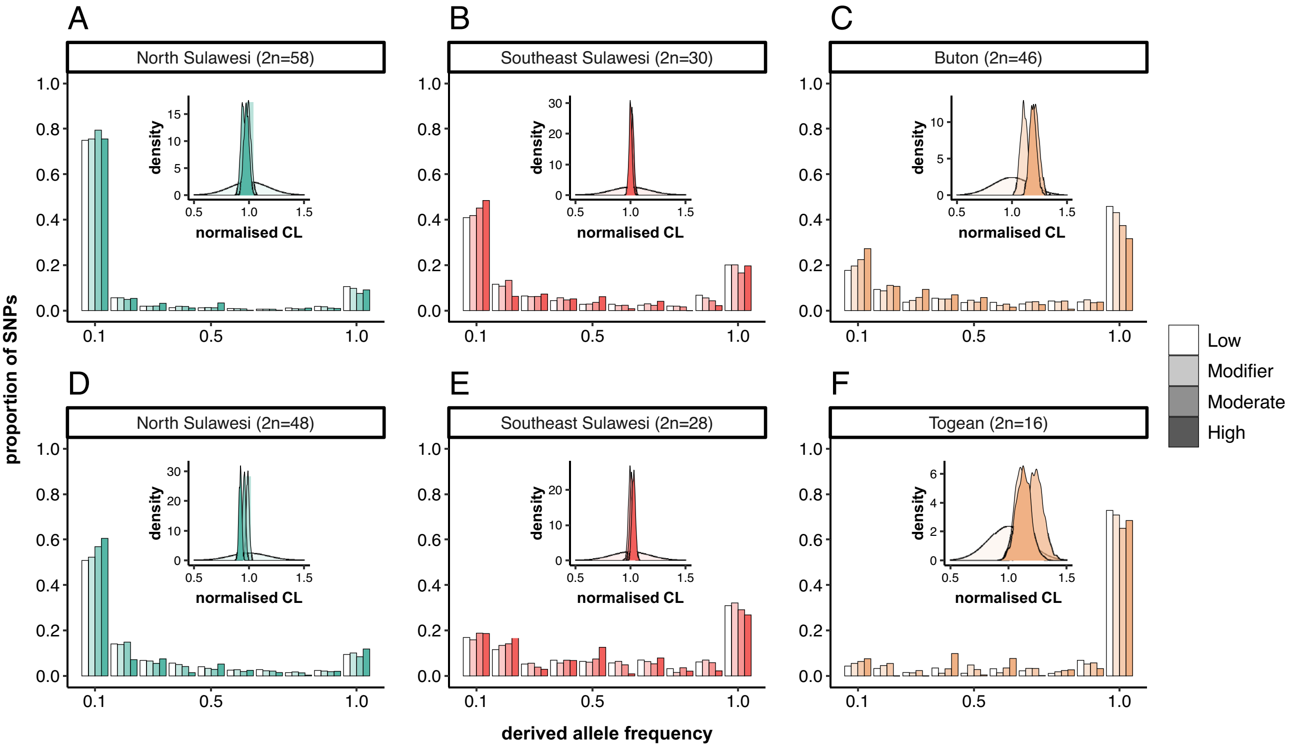

Fig. 4. SFS of deleterious alleles across anoa and babirusa populations. Site frequency spectra of alleles with four different impact ratings (low, modifier, moderate, high) across populations of anoa (*A*–*C*) and babirusa (*D*–*F*) with a corresponding 1000 bootstrap of CL values within each SFS graph representing differences between the shape of the SFS across the four impact ratings using low impact rating as the expected likelihood.

Conclusions

Our analyses show that anoa and babirusa populations on small islands have remained sufficiently stable to efficiently purge deleterious alleles. These populations also occupy high-quality habitats, often within protected areas (e.g., 45% of the Togean islands are protected as a national park). Small-island ecosystems may thus offer a long-term solution to preserve these species. In contrast, we show that some populations on the larger island of Sulawesi, such as in the Southeast, occupy lower quality habitat due to a higher degree of anthropogenic disturbance and show signs of strong bottlenecks and weaker purifying selection. These findings imply that recent anthropogenic disturbances on larger islands may be reshaping the extinction dynamics during the Quaternary, a period during which populations on smaller islands have been generally presumed to be more vulnerable. Our results suggest a possible reversal of this trend, linked to greater human impact which could make large island populations more susceptible to extinction than their smaller island counterparts.

The introduction of individuals from the mainland could be a solution to increase genetic diversity on small islands with high habitat quality and low anthropogenic pressures. Our genomic data, however, indicate that individuals from mainland populations possess more deleterious alleles, which, if translocated to smaller, less heterozygous, island populations could result in fitness decline and increased risk of extinction (11). We therefore suggest that, unless the census population size of babirusa and anoa drops dramatically on smaller islands, conservation efforts should focus on maintaining forest habitat, without the need for a possibly counterproductive, and logistically challenging, translocation program. Translocations, however, could become useful in the future and for other species; for example, in the event that populations drop dramatically, or if fixed deleterious alleles—which are more frequent in smaller island populations (Fig. 4)—start impacting fitness (31). Altogether, our study demonstrates the benefit of combining genomic information with species distribution modeling to help predict future anthropogenic threats and inform species conservation planning for island systems.

Materials and Methods

Samples used for this study were obtained from a previous study (16) and totaled 67 anoa (29 from North Sulawesi, 15 from Southeast Sulawesi, and 23 from Buton) and 46 babirusa (24 from North Sulawesi, 14 from Southeast Sulawesi, and 8 from Togean). For details of the samples, see *SI Appendix*. DNA was extracted from hair follicles or skeletal remains using DNeasy Blood and Tissue kits (Qiagen) with the final extract eluted in 100 μL of TE buffer. Double-indexed standard illumina libraries were built by Novogene (in 2020) or Macrogen (in 2021) (Dataset S1). Libraries were pooled equimolarly and sequenced on an Illumina Novaseq S4 platform (150 bp PE). To compare with other taxa that are closely related, we downloaded 25 genomes from European Nucleotide Archive (ENA) and 26 genomes from Sequence Read Archive comprising 8 species of Suidae and 10 species of Bovidae (Dataset S2) that were also sequenced using Illumina (150 bp PE). Each paired-end fastq files sample was trimmed with AdapterRemoval (32) and aligned to the using the BWA MEM (33) to a closely related reference genome, i.e., water buffalo and babirusa, and distantly related reference genome, i.e., cow and pig, for anoa and babirusa, respectively, constructing each to a set of close relative alignment and distant relative alignment (*SI Appendix*, *Supplementary Text*). Other than the population structure and genetic load analyses that use the distant relative alignment to get gene annotation information, all downstream analyses were conducted using the close relative alignment. Only genomes with mean reads depth of at least 5× were analyzed. Habitat suitability models were generated using anoa and babirusa occurrence data and environmental covariates (*SI Appendix*, *Supplementary Text*). Ensemble distribution models (21) were generated and analyzed in R (34) and QGIS (35). Further details for computational analyses used in this study are provided in *SI Appendix*.

Data, Materials, and Software Availability. All sequences used in this study are stored in ENA as BioProject PRJEB50394 (36). All codes used for analyses in this paper is openly available in GitHub (https://github.com/sagitaninta/Wallacea) (37). All other data are included in the article and/or *SI Appendix*.

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