Unexpected Island Effects at an Extreme: Reduced Y Chromosome and Mitochondrial DNA Diversity in Nias

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Abstract

The amount of genetic diversity in a population is determined by demographic and selection events in its history. Human populations which exhibit greatly reduced overall genetic diversity, presumably resulting from severe bottlenecks or founder events, are particularly interesting, not least because of their potential to serve as valuable resources for health studies. Here, we present an unexpected case, the human population of Nias Island in Indonesia, that exhibits severely reduced Y chromosome (non-recombining portion of the Y chromosome [NRY]) and to a lesser extent also reduced mitochondrial DNA (mtDNA) diversity as compared with most other populations from the Asia/Oceania region. Our genetic data, collected from more than 400 individuals from across the island, suggest a strong previously undetected bottleneck or founder event in the human population history of Nias, more pronounced for males than for females, followed by subsequent genetic isolation. Our findings are unexpected given the island's geographic proximity to the genetically highly diverse Southeast Asian world, as well as our previous knowledge about the human history of Nias. Furthermore, all NRY and virtually all mtDNA haplogroups observed in Nias can be attributed to the Austronesian expansion, in line with linguistic data, and in contrast with archaeological evidence for a pre-Austronesian occupation of Nias that, as we show here, left no significant genetic footprints in the contemporary population. Our work underlines the importance of human genetic diversity studies not only for a better understanding of human population history but also because of the potential relevance for genetic disease–mapping studies.

Key words: Nias, Austronesian, human genetic diversity, population bottleneck, Y chromosome, mitochondrial DNA.

Introduction

The Indonesian island of Nias is located at the western fringe of Island Southeast Asia, approximately 120 km off the coast of Sumatra (fig. 1). With a surface of 5,450 km^2 (Duha and Telaumbanua 2004), it is the largest of a chain of islands known as the Barrier Islands stretching along the Sumatran coast from Enggano in the south to Simeulue in the north and extending further northwards to the Nicobar and Andaman Islands. The inhabitants of Nias, or "ono niha," numbered 678,347 in the year 2000, and are sometimes considered a separate ethnic group distinct from other Indonesian tribes (Kleiweg de Zwaan 1914; Loeb 1935; Beatty 1993). Although Indonesia is primarily Muslim, the people of Nias are mostly Christians. Nias is well known for the unique architecture of its traditional houses and its megalithic monuments, which has given rise to speculations about the origins of its people (Mulia 1981; Partanda Koestoro and Wiradnyana 2007; Bonatz 2009). The Nias language belongs to the western (region) of the Malayo-Polynesian language branch within the Austronesian language family (Brown 2001; Ethnologue 2009). However, some linguists have suggested that it retains elements of an earlier eastern Austronesian language group (Nothofer 1986, 1994; Mahdi 1988). Interestingly, the Nias language possesses some phonological/grammatical features that are unusual in the region (Brown 1997, 2005). Although similarities with surrounding languages do exist at a broader level, the precise relationship between the Nias language and other Austronesian languages of Southeast Asia is still unclear. Notably, a recent study presented a consensus phylogenetic tree of 400 Austronesian languages in which Nias occupies a deep-rooted branch without any close neighbors (Gray et al. 2009). Within Nias, there are several dialects, with a broad division between northern and southern parts of the island (Beatty 1993), and along with other cultural differences between these two parts, there also is a political boundary between south Nias and the rest of the island. Socially, the people of Nias are organized into patrilineal descent groups or clans characterized by exogamous marriage with patrilocal residence, that is, a man must marry a woman from a different clan and the wife moves to the village of the husband's family. In the south of Nias, there exists an

Fig. 1. Map showing the location of Nias Island within Island Southeast Asia.

additional layer of inherited social complexity: the nobles (*Si'ulu*) and the commoners (*sato*). The *Si'ulu* can be considered an endogamous group in the sense that only individuals both of whose parents are *Si'ulu*, become *Si'ulu* themselves. However, it is common for *Si'ulu* men to have secondary marriages with non-*Si'ulu* women, and offspring from such a union would not inherit *Si'ulu* status; consequently, male gene flow from *Si'ulu* to *non-Si'ulu* can be expected to occur, whereas *Si'ulu* female gene flow would be expected to remain restricted to *Si'ulu* members.

In recent centuries, Nias has been under the influence of various foreign powers. In the 17th century, Nias chiefs were known to be trading slaves for gold from the Acehnese of Sumatra (Ricklefs 1981), and it is reported that West Sumatran, Chinese, and European slave traders were also active in Nias (Partanda Koestoro and Wiradnyana 2007). The British East India Company had maintained trading interests in Nias from before the close of the 17th century until 1824 when British interests in Sumatra were ceded to the Dutch by the Treaty of London (Bastin 1965). The Dutch had also established a small trading post in the north of Nias before the end of the 17th century, although they did not have administrative control of the island until it was granted to them by the 1824 treaty. The slave trade continued during British and Dutch times and was not brought to an end until the 20th century (Beatty 1993). It is claimed that the proportion of Niasans lost to the slave trade annually between 1790 and 1830 was approximately 0.4% of its population or 800 to 1,500 inhabitants (Reid 2004). From the early 18th century missionaries, mostly from Germany, arrived in Nias; Christianization went on slowly and continued into the 20th century (Beatty 1992). During the Second World War, the Japanese occupied the island, and in 1945, Nias became part of the newly founded Republic of Indonesia.

Despite a long-standing anthropological interest in the Nias culture, surprisingly little is known about the earlier history of the Nias people and where they came from. Hummel and Telaumbanua (2007) recently summarized the diverse views on potential homelands of the Niasans, including North Sumatra, Burma, Northeast India, and southern China. Some linguists (Mahdi 1988; Nothofer 1994) have claimed that the current Niasans may represent survivors of an early offshoot of the Austronesian expansion, a notion also supported by the most recent linguistic analyses (Gray et al. 2009). However, it has been argued that the rich cultural diversity present in Nias is suggestive of multiple immigration waves (Mulia 1981; Hummel and Telaumbanua 2007). Recent archaeological excavations in the Tögi Ndrawa cave pointed to a Paleolithic presence of modern humans in Nias at about 12,000 years ago (Hämmerle 1999, 2009; Forestier et al. 2005), hence much before the regional Austronesian arrival assumed to be around 4,000–5,000 years ago (Bellwood 2005; Gray et al. 2009). However, so far it is unclear to what extent, if at all, these early inhabitants have contributed to the gene pool of the current population of Nias. Given that the current language of Nias is Austronesian, it can be hypothesized that the islanders should trace a considerable part of their genetic ancestry, to the Austronesian occupation; however, the archaeological findings from pre-Austronesian times may expect at least some non-Austronesian ancestry as well. Thus far, there has been no comprehensive genetic study of Nias islanders, which might throw new light on, or possible answers to, the various competing hypotheses suggested by the linguistic, anthropological, and archaeological data. Limited human genetic data, restricted to the Y chromosome, are available as part of two studies that looked at wider geographic regions and did not specifically address the human history of Nias (Li et al. 2008, Karafet et al. 2010). To explore the genetic diversity and to shed light on the genetic history of Nias islanders, we investigated paternally inherited Y chromosome (NRY) and maternally inherited mitochondrial DNA (mtDNA) polymorphisms in >400 individuals from various locations within the island and also compared these data with those of a large number of other populations from the Asia/Oceania region.

Materials and Methods

Samples

Samples were collected with informed consent and belong to the following groups: Si'ulu ($n = 77$), Fau ($n = 47$), and Sarumaha ($n = 12$) in South Nias; Hia ($n = 92$), Ho ($n = 21$), Daeli ($n = 32$), Laoya ($n = 8$), Zebua ($n = 30$), Hulu ($n = 4$), Zalukhu ($n = 12$), and Gözö ($n = 42$) in North Nias; as well as a rest group of unassigned individuals from diverse locations within the island ($n = 67$). Extensive questionnaire information was collected from the participants, which served to exclude from our study potentially related individuals up to the grandparent level. The procedure has been approved by the Nias Government through Nias Health Office (Reference No. 443/3898/P2P, June 3rd 2002) and by the ethics committee of the University of Münster (protocol No. 3XKenn1); genetic work was carried out under the approval of the Erasmus MC ethics committee.

Reference data used in this study were previously genotyped and described (Kayser et al. 2006, 2008; Mona et al. 2007) and include populations from East Asia (South Korea, China, Taiwan Chinese, and Taiwan Aborigines), Southeast Asia (Vietnam, Malaysia, Philippines, Borneo-Barito river area, Sumatra-Riau Province, Java, Nusa Tenggara, and Moluccas), Near Oceania (Baham, Karon, Mai Brat, and Ekari from northwest New Guinea; Dani/Lani, Una, Ketengban, Citak, and Asmat from southwest New Guinea; and Admiralty Islands, New Britain Tolai, Trobriand Islands, Bereina, Kapuna, and coast and highlands from Papua New Guinea), Remote Oceania (Fiji, Futuna, Tuvalu, Tonga, Samoa, Niue, and Cook Islands), and Australia (Arnhem Land and Sandy Desert). In addition, we included in our reference data set a sample from the Karo Batak of North Sumatra ($n = 23$), not previously described.

Genotyping

A prescreening of a selected subset of Nias samples using a Y-single nucleotide polymorphism (SNP) multiplex system targeting the major worldwide NRY haplogroups revealed that 100% of this subset belonged to haplogroup O-M175 (data not shown). Subsequently, all male samples were genotyped at 14 selected Y-SNPs that characterize haplogroup O-M175 and its sublineages (M175, M119, M101, M110, M268, M95, M88, M122, M324, M121, M164, M159, M7, and M134) using an in-house multiplex snapshot assay (see Kayser et al. 2008 for primer details). Seventeen Y-short tandem repeats (STRs) were genotyped using the AmpFlSTRYfiler Kit (Applied Biosystems) following the manufacturer's recommendations. mtDNA data were generated by sequencing the first hypervariable segment (HVS1), corresponding to position 16024–16392 of the control region, as described previously (Kayser et al. 2006), and were deposited in GenBank under accession numbers GU455526–GU455965. A subset of the Nias samples not assignable unequivocally to mtDNA haplogroups based on the HVS1 sequence data alone was subjected to restriction fragment length polymorphism screening for mtDNA coding–region position 4850 (which defines a subclade of mtDNA haplogroup M7) using polymerase chain reaction amplification primers 5′-AAGCAACCGCATCCATAATC-3′ and 5′-ATTTTGCGTAGCTGGGTTTG-3′ and restriction enzyme HaeIII.

Data Analysis

NRY haplogroups were inferred based on the Y chromosome SNP tree by Karafet et al. (2008). mtDNA HVS1 sequences were aligned to the revised Cambridge Reference Sequence (rCRS) (Andrews et al. 1999), and variant nucleotide positions (np) were scored accordingly. A to C transversions at positions 16181–16183 were disregarded because of their dependence on the transition at position 16189. mtDNA haplotypes were classified into their respective haplogroups using the most recent mtDNA phylogeny available at http://www.phylotree.org (van Oven and Kayser 2009). Median-joining haplotype networks (Bandelt et al. 1999) were constructed using the program Network version 4.5.1.6 (http://www.fluxus-engineering.com). Y-STR loci were weighted according to their individual mutation rates (Goedbloed et al. 2009), by applying a 5-fold weighting scheme with higher weights given to slowly evolving markers and lower weights to faster evolving marker. The multicopy marker DYS385 was not used because its alleles cannot be unambiguously assigned to their respective loci with the genotyping system applied. The complex marker DYS389II was excluded because, even when DYS389I is subtracted from it, it contains multiple polymorphic substretches in combination with a relatively high mutation rate, so that seemingly identical alleles can have a different underlying substructure (Forster et al. 2000; Zhivotovsky et al. 2004). Furthermore, the four highest mutating Yfiler loci (DYS439, DYS456, DYS458, and DYS635) as established recently (Goedbloed et al. 2009) were excluded to reduce network complexity. The remaining ten Yfiler Y-STR loci (DYS19, DYS389I, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS448, and Y-GATA-H4) were used for all within-Nias analyses. Analyses that included reference populations were based on a subset of six of these loci (DYS19, DYS389I, DYS390, DYS391, DYS392, and DYS393) that were genotyped in the entire data set. Population diversities, genetic distance measures ($F_{ST}/R_{ST}/\Phi_{ST}$), and analysis of molecular variance (AMOVA) were calculated using Arlequin 3.11 (Excoffier et al. 2005). Multidimensional scaling (MDS) was performed with the ALSCAL algorithm available in SPSS statistical software (SPSS Inc., Chicago, IL).

Pairwise comparisons of population diversities were evaluated for statistical significance using a custom-made script in R (http://www.R-project.org). Haplotype frequencies of each population were estimated using a Bayesian approach. Under the assumption of no prior knowledge about the haplotype frequencies and assuming a multinomial model for the observed haplotype counts, a posterior Dirichlet distribution of the haplotype frequencies was estimated (Gelman et al. 1995). From each Dirichlet posterior distribution, the haplotype frequencies were resampled, and from these haplotype frequencies, the expected heterozygosity was computed; this process was iterated 10,000 times, and for each iteration, it was determined whether the Nias population had a diversity lower than that of the population it was compared with; the fraction of iterations in which this was not the case provided the P value.

Results

NRY Variation in Nias

Y-SNP typing revealed basically only two NRY haplogroups among 407 Nias males. Nearly 70% of Niasans belonged to haplogroup O-M119*(xM101,M110) and 30% belonged to haplogroup O-M110, besides a single male of haplogroup O-M95*(xM88) (supplementary table S1a, Supplementary Material online). No other Y chromosomes, including none of potential European ancestry, were detected. The distribution of NRY haplogroups within Nias was highly skewed,

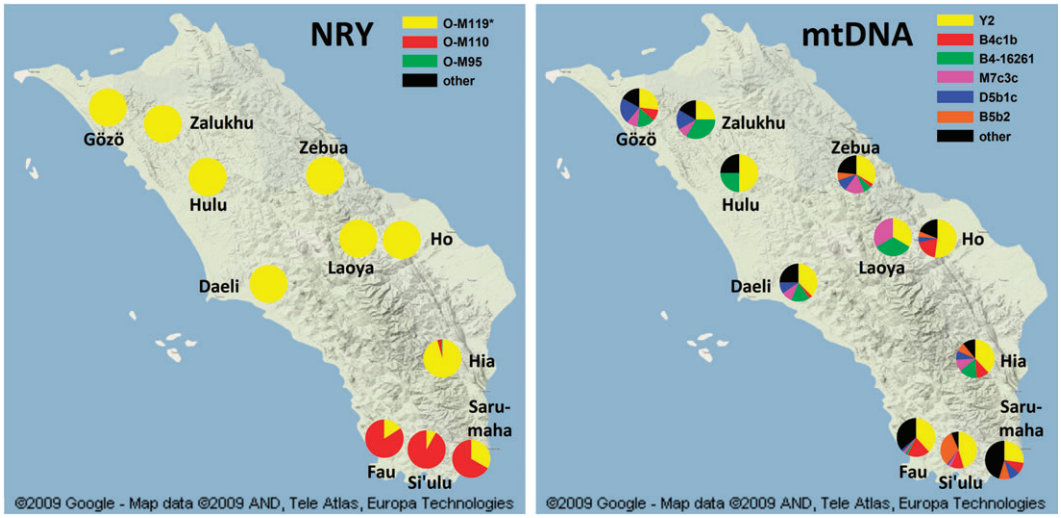

Fig. 2. Distribution of NRY and mtDNA haplogroups within Nias. For mtDNA, only haplogroups with an overall frequency in Nias of >5% are shown, whereas the remaining minor haplogroups are included as "other." The underlying data for this figure can be found in supplementary table S1a (Supplementary Material online).

with haplogroup O-M110 only observed in South Nias and in high frequency, whereas O-M119* was predominantly found in North Nias (fig. 2). At a resolution of ten Y-STR loci, the 407 Nias Y chromosomes fell into only 41 different haplotypes (here designated y1–y41) (supplementary table S1b, Supplementary Material online), with 14 haplotypes belonging to haplogroup O-M110 and 26 haplotypes to O-M119*. The positioning of the ten-locus Y-STR haplotypes in a median-joining network was in agreement with the haplogroup affiliations as determined from Y-SNP typing (fig. 3). The O-M119* cluster showed a clear star-like structure with haplotype y9 as the most central and most frequent haplotype, making it a likely founder haplotype for Nias O-M119* Y chromosomes. For the O-M110 cluster, however, the majority of individuals belonged to two closely related haplotypes, y27 and y28. Haplotypes y35–38 and y40 formed a separate cluster at some mutation step distance from the O-M110 core haplotypes, potentially reflecting an intrahaplogroup subdivision. In line with this observation, the within-haplogroup haplotype diversity was significantly higher for O-M110 (0.78 ± 0.02) than for O-M119* (0.58 ± 0.03) (fig. 3). A 2D MDS plot based on R_{ST} values from Y-STR haplotypes (fig. 4A) showed a clear separation between the groups from North Nias and those from South Nias as a result of their association with the two NRY haplogroups. AMOVA based on Y-STR haplotypes revealed that a large proportion of 60.7% ($P < 0.01$) of the total genetic variance is explained by grouping the population samples into North and South Nias.

mtDNA Variation in Nias

Among the 440 HVS1 mtDNA sequences, only 67 different haplotypes (here designated mt1–mt67) were observed (supplementary table S1c, Supplementary Material online). They were classified into 18 different mtDNA haplogroups that could be distinguished on the basis of HVS1 polymorphisms in combination with additionally collected data from one coding-region SNP (np 4850) (supplementary table S1a, Supplementary Material online). This includes one novel haplogroup, characterized by a distinctive 16246T transversion, which, in the absence of complete sequence data, we tentatively refer to as M-16246T. The six mtDNA haplogroups with overall population frequencies exceeding 5% were Y2 (40.0%), B4c1b (10.2%), B4-16261 (9.3%), B5b2 (9.1%), M7c3c (8.0%), and D5b1c (7.1%). The distribution of these haplogroups within Nias is shown in figure 2. Haplogroup B4-16261 was found virtually absent from southern Nias; in contrast, haplogroup B5b2 was absent from the most northern groups in Nias (Gözö, Zalukhu, and Hulu) but had a relatively high frequency (32.5%) in the southern *Si'ulu*. Haplogroup Y2 was observed with high frequency everywhere in Nias. Overall, the mtDNA picture of haplogroup distribution appeared much less extreme than the NRY haplogroup situation where a strong north–south difference was observed. mtDNA haplotype networks for the six major haplogroups in Nias are shown in supplementary figure S1 (Supplementary Material online). Notably, haplogroup B5b2, present mainly in the *Si'ulu*, consists of only a single HVS1 haplotype, whereas the other five haplogroups are represented in Nias with six to ten different HVS1 haplotypes. A 2D MDS plot based on Φ_{ST} values from mtDNA HVS1 sequences (fig. 4B) did not show a similarly extreme north–south separation as observed for NRY. Moreover, AMOVA based on mtDNA showed that a north/south grouping explained only 4.5% ($P = 0.04$) of the total variance (as opposed to 60.7% for NRY).

Comparing NRY/mtDNA Distribution with Asia/Oceania

To explore population relationships of Nias, we have analyzed the NRY/mtDNA haplogroups found in Nias in the context of a large number of reference populations from East Asia, Southeast Asia, Near and Remote Oceania,

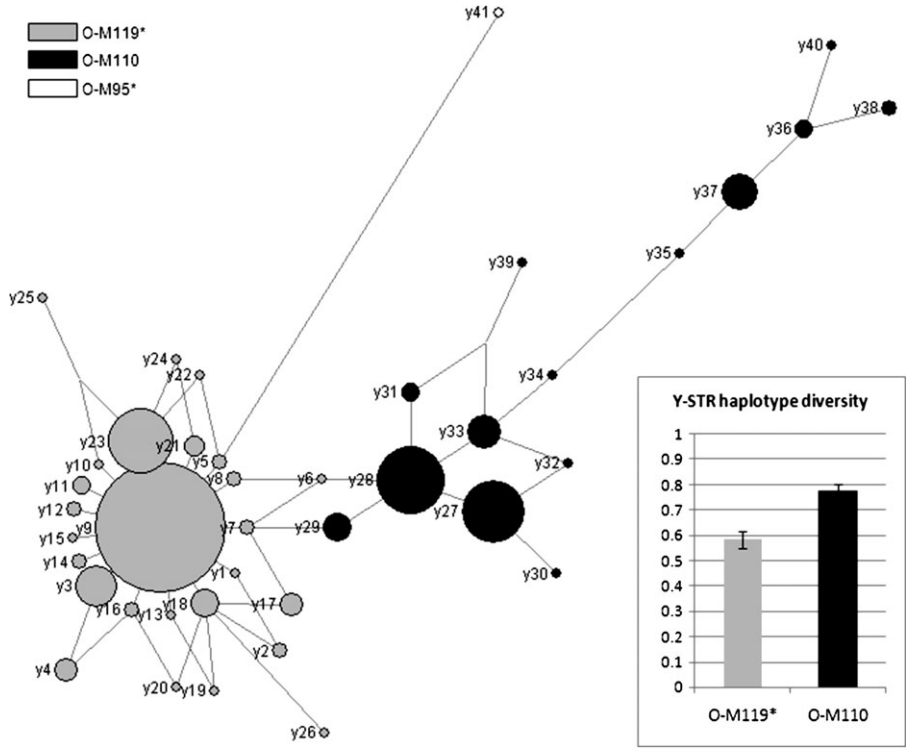

Fig. 3. Median-joining haplotype network connecting 41 different ten-locus Y-STR haplotypes detected in Nias, representing a total of 407 males. Circle sizes are proportional to the number of sampled carriers. Gray, black, and white circle fillings indicate haplogroup status. Node names correspond to the haplotype IDs in supplementary table S1b (Supplementary Material online). The insert bar chart shows haplogroup-associated Y-STR haplotype diversity for the two major haplogroups.

and Australia (fig. 5 and supplementary table S2a and b, Supplementary Material online). Most notably, NRY haplogroup O-M110, which in Nias was only found in the south and at a very high frequency of 86.4%, was present elsewhere in our reference populations at much lower frequencies. Being completely absent from mainland East Asia, O-M110 was found with appreciable frequencies in some parts of island East/Southeast Asia, such as in Taiwan Aborigines (32.6%) and Philippines (12.8%), but only sporadically in other parts of Southeast Asia, such as Java, Borneo, and the Moluccas. Notably, this haplogroup was completely absent from Sumatra (Riau and Karo Batak) and Malaysia, the geographically nearest populations to Nias, as well as from Nusa Tenggara. In Near Oceania, O-M110 was observed with appreciable frequencies only in two regions, the Admiralty Islands (17.6%) and the Trobriand Islands (17.0%); in Remote Oceania it was virtually absent with only sporadic observations in Fiji and Tuvalu. The situation was different for NRY haplogroup O-M119*, which was widely present throughout much of Southeast Asia, reached a frequency of 46.5% in Taiwan Aborigines and also occurred in mainland East Asia. However, when moving into Near Oceania, the frequency of O-M119* dropped drastically, only occurring in the Trobriand Islands (11.3%) and in a single Papua New Guinea Highlander, and this haplogroup was also mostly absent from Remote Oceania, except sporadic observations in Fiji and Samoa.

Supplementary figure S2 (Supplementary Material online) shows median-joining networks relating six-locus Y-STR haplotypes for haplogroup O-M119* and O-M110 from Nias as well as the reference populations. In both cases, the haplotypes that were most frequent in Nias were shared with a variety of other populations, so that, at this level of Y-STR resolution, there was no clear information obtainable from the networks where the Nias haplotypes may have originated geographically. In a 3D MDS plot based on NRY haplogroup derived F_{ST} values (fig. 6A), in which we included North and South Nias as separate populations (because of the strong NRY haplogroup differences), both groups took outlier positions, away from their geographically neighboring populations, and in dimension 3 were also at large distance from each other.

A comparison of the Nias mtDNA data with that of our regional reference populations revealed that the dominant mtDNA haplogroup in Nias, Y2, with an overall frequency of 40.0%, was present only sporadically in a few East and Southeast Asian populations (China, Malaysia, Philippines, Sumatra [Riau and Karo Batak]) but absent in all other groups studied. Haplogroup B4c1b, with 10.2% frequency in Nias, was most frequent in Java (14.6%), and also present at considerable frequencies in Vietnam (9.4%) and in the Karo Batak from Sumatra (8.7%), but was rare in other Southeast Asian groups and absent from mainland East Asia as well as from Near and Remote Oceania. Haplogroup B4-16261, with a frequency in Nias of 9.3%, occurred throughout East Asia, Southeast Asia, as well as Near and Remote Oceania with highest frequencies in the Trobriand Islands in Near Oceania (25.0%), as well as in Tuvalu

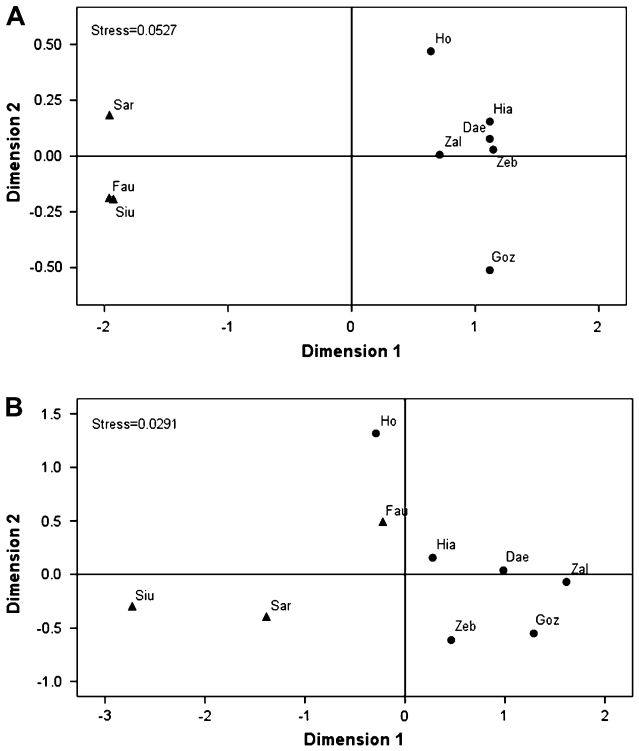

FIG. 4. MDS plots based on Y-STR derived R_{ST} values (*A*) and mtDNA HVS1 sequence derived Φ_{ST} values (*B*). Groups with a sample size <10 were omitted. The population codes are as follows: Siu for Si'ulu, Fau for Fau, Sar for Sarumaha, Goz for Gözö, Zeb for Zebua, Dae for Daeli, Hia for Hia, Ho for Ho, and Zal for Zalukhu. Groups from South Nias are indicated with triangles and those from North Nias with circles.

(25.4%) and Tonga (20.5%) in Remote Oceania. Haplogroup B5b2, with a frequency of 9.1% in Nias, was only found at low frequencies in the Karo Batak from Sumatra (4.3%) and Korea (4.2%) and absent from all other groups studied. Haplogroup M7c3c, with a frequency in Nias of 8.0%, was present at moderate frequencies in East and Southeast Asia with its highest frequency in Borneo (14.3%) but absent east of the Admiralty Islands except for a single individual from Tuvalu in Remote Oceania. Haplogroup D5b1c had a frequency of 7.0% in Nias and was found in a single individual from the Moluccas but was completely absent from all other groups studied. The 3D MDS based on mtDNA haplogroups (fig. 6B) showed a clear correlation with geography in the first two dimensions, with Nias clustering with the East and Southeast Asian populations; however, in dimension 3, Nias appeared clearly separated from the main cloud of population data points.

Comparing NRY/mtDNA Diversity with Asia/Oceania

In addition, we compared measures of overall NRY and mtDNA haplogroup and haplotype diversity of the Nias population with those from our reference populations from East Asia, Southeast Asia, Near and Remote Oceania, as well as Australia (figs. 7 and 8 and supplementary table S3, Supplementary Material online). Notably, Nias' unbiased six-locus Y-STR diversity was, with 0.524 (±0.029), strikingly lower than that of all our reference populations (fig. 7), including Remote Oceanians and Australian Aborigines, and the differences were statistically significant ($P < 0.05$) (except for the Ekari and the Ketengban, two isolated groups from West New Guinea). For NRY haplogroup diversity (which may be influenced by SNP ascertainment bias), the picture was broadly similar, although some isolated groups from Near and Remote Oceania had lower haplogroup diversities than Nias.

In the case of mtDNA HVS1 haplotypes, Nias' diversity was, with 0.9110 (±0.0085), lower than that of East and Southeast Asian populations (fig. 8), although the differences were only statistically significant ($P < 0.05$) for some of the groups (i.e., China, Vietnam, Sumatra-Riau, Java, and Nusa Tenggara), whereas it was higher than that of most Near Oceanic (except for Kapuna and highlands in Papua New Guinea) and all Remote Oceanic populations. For mtDNA haplogroups, the picture was very similar, although standard deviations were larger.

Discussion

Paternal Genetic History of Nias

In this study, we have documented a strongly reduced male-specific genetic diversity in Nias. In our sample of 407 Nias Y chromosomes, basically only two haplogroups were observed: O-M119*(xM101,M110) and O-M110. The frequency of O-M119* in Nias is the highest of the entire Asia/Oceania region known thus far. Haplogroup O-M119* is assumed to be of Neolithic East Asian, most likely Austronesian, origin (Kayser et al. 2001), and is common across East and Southeast Asia but rare in Near and Remote Oceania where it may have been lost due to genetic drift (Capelli et al. 2001; Scheinfeldt et al. 2006; Kayser et al. 2008; Li et al. 2008) (fig. 5). The other NRY haplogroup in Nias, O-M110, is assumed to have originated in Taiwan (Kayser et al. 2008) and, like O-M119*, has been proposed as a signature of the Austronesian expansion into Southeast Asia and Near Oceania, although in Remote Oceania it mostly got lost by genetic drift (Kayser et al. 2008). O-M110 so far has not been detected on the East Asian mainland, and within Southeast Asia is much rarer than O-M119*. The high frequency of O-M110 in Nias is intriguing because we did not detect O-M110 at all in the direct geographic neighbors Sumatra (Riau and Karo Batak) and Malaysia (fig. 5) and only sporadically in the more distant neighbors Borneo, Java, and Nusa Tenggara. This pattern seems to suggest that keeping with a Taiwanese origin, the southwestward spread of males carrying O-M110 Y chromosomes from Taiwan via the Philippines occurred only at very moderate frequency and/or that O-M110 Y chromosomes were subsequently lost by genetic drift in many other parts of Southeast Asia. The high frequency of O-M110 in Nias is most likely a result of a bottleneck/founder effect together with subsequent isolation. This view is supported by the overall O-M110 Y-STR network (supplementary

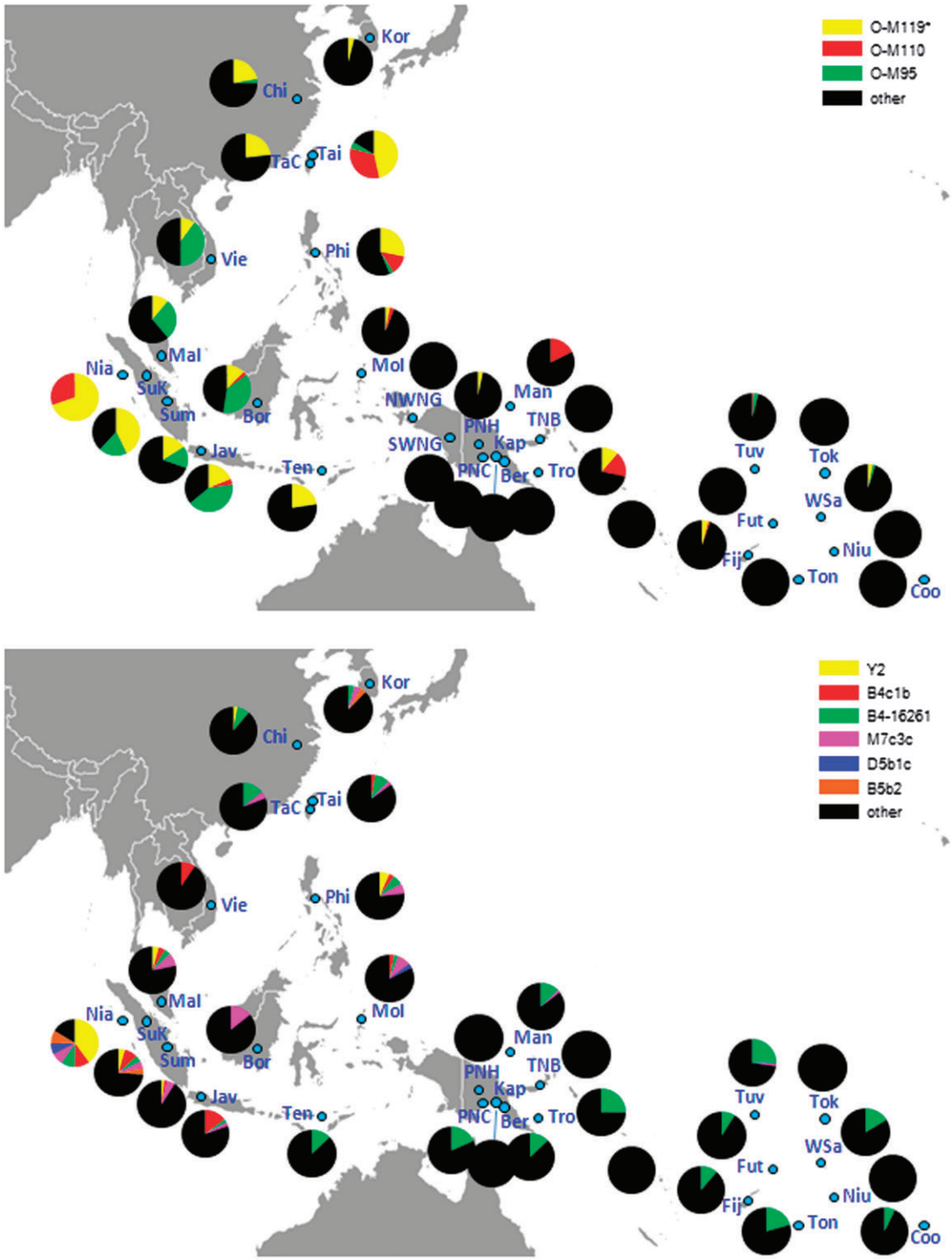

Fig. 5. Regional distribution of those Y (upper) and mtDNA (lower) haplogroups that occur in Nias. For mtDNA, only haplogroups with an overall frequency in Nias of >5% are specified, the remaining haplogroups are included as "other." The underlying data for this figure can be found in supplementary table S2a and b (Supplementary Material online).

fig. S2, Supplementary Material online) in which Niasan haplotypes are mostly shared with those of other populations but also show a Nias-specific component. Interestingly, the frequency pattern of O-M110 in Southeast Asia is similar to that in Near Oceania, where O-M110 is also generally rare but reaches high frequencies (although not as high as in Nias) in some island populations, such as the Trobriand and Admiralty Islands (Kayser et al. 2008). However, an alternative view has also been proposed recently, namely, that O-M110 and O-M119*/P203 (see below) in Southeast Asia possibly represent a pre-Austronesian dispersal (Karafet et al. 2010).

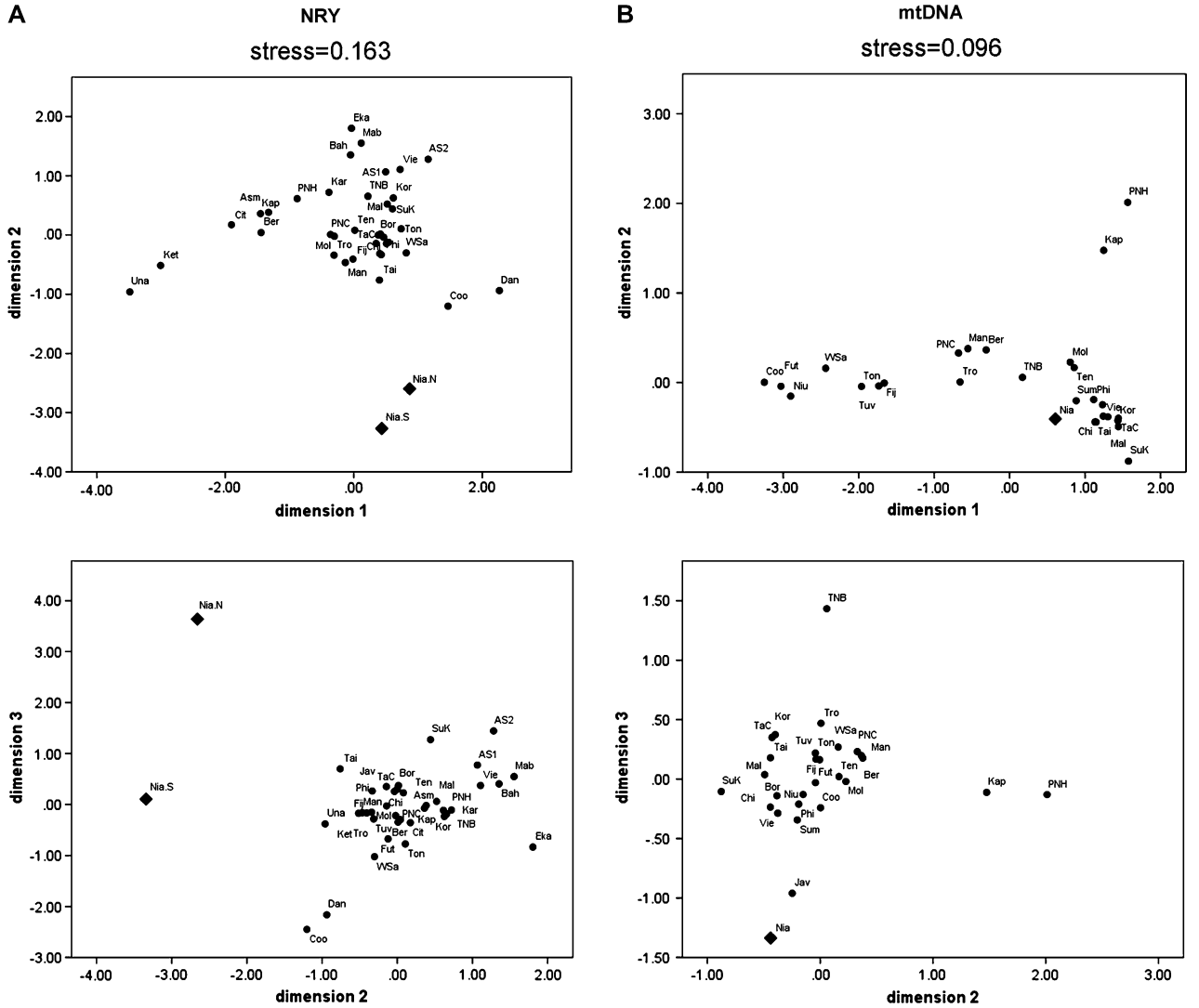

FIG. 6. MDS plots based on NRY (*A*) and mtDNA (*B*) haplogroup derived F_{ST} values. Population codes can be found in supplementary table S3 (Supplementary Material online). Data points referring to Nias are indicated with diamonds and other populations with circles. NRY haplogroups of likely European origin (F-M89, P-M74, and R-M173) were excluded for F_{ST} calculation.

Moreover, the distribution of the NRY haplogroups within Nias is quite remarkable: Whereas haplogroup O-M119* occurs everywhere in Nias and represents the only NRY haplogroup we observed in North Nias, haplogroup O-M110 is restricted to South Nias, where it occurs at very high frequency (86.4% for the three southern groups Si'ulu, Fau, and Sarumaha combined) (fig. 2). Notably, intrahaplogroup Y-STR haplotype diversity is higher for O-M110 than for O-M119* (fig. 3), suggesting that within Nias, O-M110 is older than O-M119*, which is the opposite of what is expected from the phylogenetic relationship of both markers because the M110 mutation occurred on the background of the M119 mutation (Karafet et al. 2008). The strong north–south differentiation in NRY diversity, statistically supported by AMOVA, is consistent with a cultural–linguistic north–south divide of the island and suggests little male gene flow across this border. The opposing male gene pools of southern versus northern Nias either reflect extreme genetic drift effects after the separation of both groups from an assumed common ancestral population or, alternatively, may be explained by different paternal source populations followed by genetic isolation. Y-STR network analysis in Nias revealed a highly star-like structure for haplogroup O-M119* lineages with one very frequent haplotype in the center (y9) surrounded by many less frequent haplotypes at one or two mutation step distance (fig. 3). This pattern is indicative of a strong bottleneck/founder effect involving O-M119* Y chromosomes followed by population expansion and subsequent genetic isolation. Interestingly, the O-M110 Y-STR network shows a different pattern with higher interhaplotype distances and more evenly distributed frequencies, suggesting that O-M110 settlers arrived in Nias with already diversified haplotypes reflecting a less severe bottleneck/founder effect as seen for O-M119* Y chromosomes or, alternatively if the lineages evolved in situ, that O-M110 Y chromosomes in Nias are older/arrived earlier than O-M119* Y chromosomes in Nias.

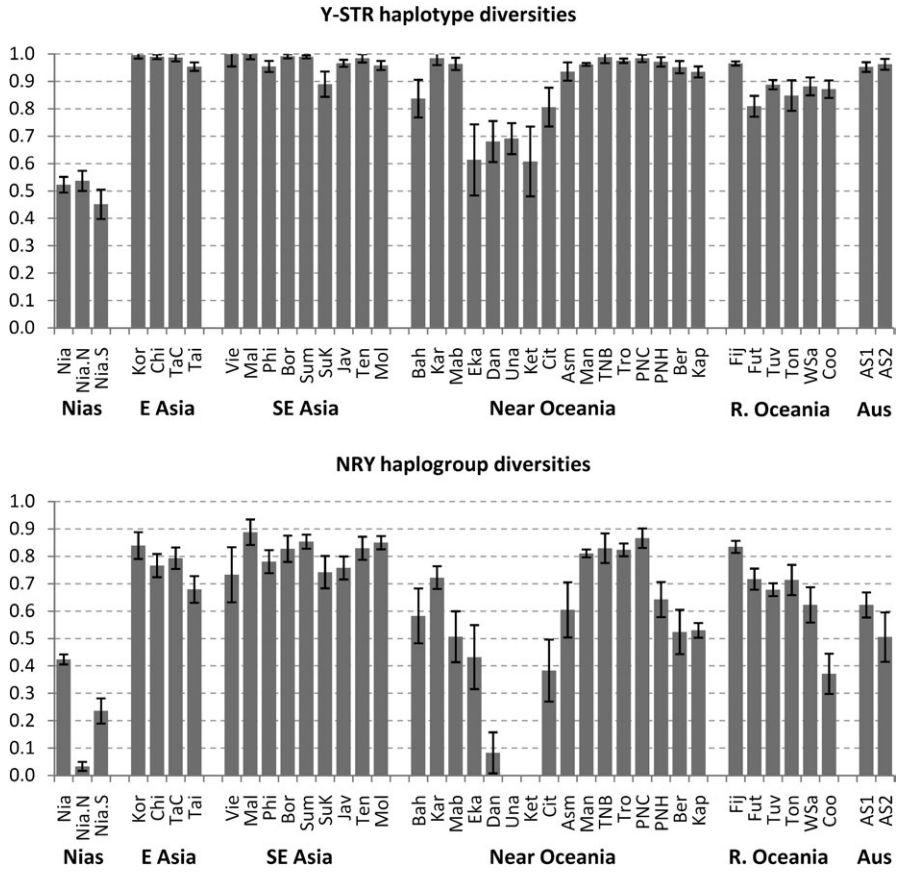

Fig. 7. Y-STR haplotype and NRY haplogroup diversities of Nias and reference populations. Error bars represent standard deviations. The underlying data for this figure and population codes can be found in supplementary table S3 (Supplementary Material online).

According to oral tradition from Nias, all clans share a single common paternal origin in the village Sifalagö Gomo (Hämmerle 2009), where currently the Hia clan still resides. The high Y chromosome differentiation we observe between northern and southern groups in Nias renders this a rather unlikely scenario, although we cannot completely rule out that opposing drift effects in the respective parts of the island have caused the observed pattern. In the NRY comparison with regional reference populations, North and South Nias did not cluster with any other populations from Asia/Oceania but rather appeared as clear Y chromosome outliers in the MDS analysis (fig. 6), providing further support for the special genetic situation of Nias regarding its patrilineal gene pool. The NRY haplotype networks (supplementary fig. S2, Supplementary Material online) unfortunately did not reveal the precise geographic origin of Nias Y chromosomes but at the same time did not rule out Taiwan and Island Southeast Asia as potential homelands. A more detailed analysis using a much larger panel of Y-STR loci may provide more detailed answers regarding the specific geographic origin of Nias islanders. Noteworthy, in our Nias data set, no NRY haplogroups of European origin were detected despite the European colonial history of Nias going back at least 200 years. Hence, we can conclude that admixture between Niasans and European males was insignificant, as opposed to many other Southeast Asian/Oceanic populations where European Y chromosomes, such as haplogroup R-M173, were detected previously (Kayser et al. 2008) (supplementary table S2a, Supplementary Material online).

Notably, a recent NRY study on Indonesia by Karafet et al. (2010), including 60 individuals from Nias, found that they all belonged to haplogroups O-P203 and O-M110. The marker P203, defining a subbranch of O-M119 and a sister branch of O-M110, was only recently identified (Karafet et al. 2008) and was therefore not included in our study. Given the Karafet et al. (2010) findings and the low haplogroup–associated Y-STR haplotype variation that we found for O-M119* in our study (fig. 3), it is likely that our O-M119* samples do in fact belong to haplogroup O-P203. However, we could not corroborate the data reported in a study by Li et al. (2008), which included 12 Niasans: 11 individuals belonged to haplogroup O-M122 and 1 individual to O-M88. Neither of these NRY haplogroups were observed in our extensive sample of >400 Niasans and also not in the 60 independent samples studied by Karafet et al. (2010), which may reflect a sampling issue in the Li et al. (2008) study.

Maternal Genetic History of Nias

The mtDNA composition of the Nias population was found to be more diverse than that of NRY, with 18 different haplogroups as inferred from HVS1 polymorphisms and one coding-region SNP (supplementary table S1a, Supplementary Material online). One major haplogroup, Y2, exists in

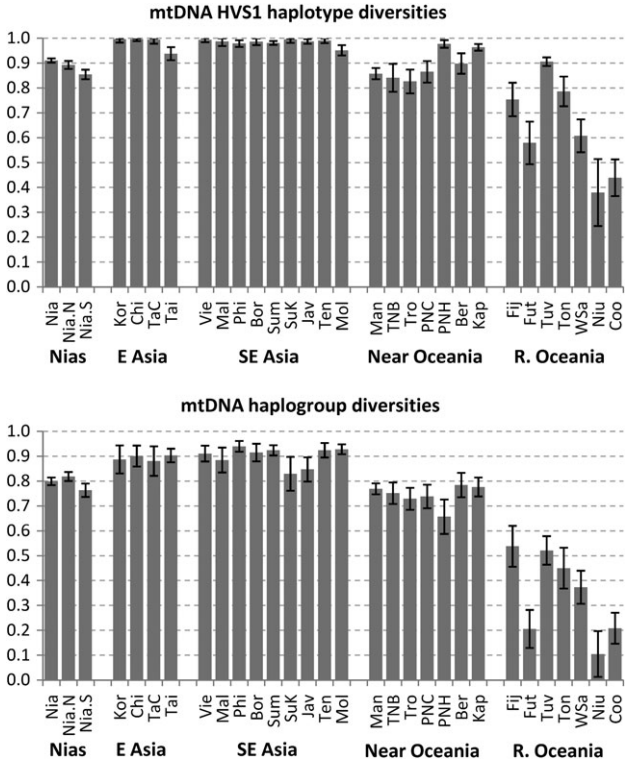

FIG. 8. mtDNA HVS1 haplotype and haplogroup diversities of Nias and reference populations. Error bars represent standard deviations. The underlying data for this figure and population codes can be found in supplementary table S3 (Supplementary Material online).

all sampled groups in Nias with an overall frequency of 40.0%. To our knowledge, this frequency is higher than previously observed for any other population studied. In our reference populations, haplogroup Y2 is only sporadically observed in China, Malaysia, the Philippines, and Sumatra (Riau and Karo Batak) (fig. 5 and supplementary table S2b, Supplementary Material online). The highest Y2 frequencies reported so far in other studies include the Philippines (12.9%), the Taiwanese Saisat tribe (9.5%), and Sumatra (combined sample from Medan, Pekanbaru, Bangka, Padang, and Palembang)) (6.7%) (Trejaut et al. 2005; Hill et al. 2007). According to Hill et al. (2007), Y2 is a plausible signal for a mid-Holocene out-of-Taiwan expansion. The five next-most abundant haplogroups in Nias (B4-16261, B4c1b, B5b2, D5b1c, and M7c3c) have similar frequencies ranging between 7.0% and 10.2%. Haplogroup B4-16261 has a wide distribution, including East and Southeast Asia as well as Oceania. Haplogroup B4c1b is present in Southeast Asia and Taiwan at relatively low frequencies. Haplogroup B5b2, which we find especially frequent among the Si'ulu in South Nias (32.5%) but rather rare in the rest of Nias, exists in our reference populations only in single individuals from Korea and Sumatra (Karo Batak). However, a previous study reported haplogroup B5b as a whole in (among others) the Philippines (9.7%), Malaysian Orang Asli (5.8%) as well as Sumatra (2.2%) and Borneo (1.3%), although not in Taiwan (Hill et al. 2007). Haplogroup D5b1c, which was first labeled D5d in Hill et al. (2007) but was later identified as a sublineage of haplogroup D5b1 and therefore relabeled D5b1c by Tabbada et al. (2010), is not present in any of our reference populations, except for a single individual from the Moluccas. Tabbada et al. (2010) reported D5b1c at very low frequency in the Philippines (0.47%). Hill et al. (2007) found haplogroup D5 as a whole at low frequencies in China, Taiwan, and in several Southeast Asian populations with highest frequency in Sulawesi (8.4%). Haplogroup M7c3c, formerly known as M7c1c (Kivisild et al. 2002; Trejaut et al. 2005; Hill et al. 2007), is present throughout East and Southeast Asia and occurs at low frequencies on the Admiralty Islands in Near Oceania and on Tuvalu in Remote Oceania. Like Y2, M7c3c was suggested as candidate haplogroup for a mid-Holocene out-of-Taiwan expansion (Hill et al. 2007). Hence, overall, all major mtDNA haplogroups in Nias are indicative of an Austronesian maternal ancestry of the current Nias population. The remaining minor mtDNA haplogroups detected in Nias, each in a frequency below 5%, include subhaplogroups of clades B4, B5, E, and F, as well as R22 and the M-16246T lineage. R22 (0.9% of Niasans) and M-16246T (1.1% of Niasans) might represent indigenous Island Southeast Asian lineages. R22 was found in the Nicobar Islands (Trivedi et al. 2006) and, within Island Southeast Asia, shows a concentration in eastern Indonesia (Hill et al. 2007). M-16246T has been detected only in a single individual from Borneo (supplementary table S2b, Supplementary Material online), in a single Singapore an Malay (Wong et al. 2007), and in single individuals from Bali, Sumba-Waingapu, and Sumatra-Pekanbaru (Hill et al. 2007). We cannot exclude the possibility that some of the very rare mtDNA lineages in Nias go back to pre-Austronesian inhabitants of the island as described from archaeological data (Forestier et al. 2005), but they might also have been picked up in Southeast Asia by Austronesian migrants on the way to Nias. In any case, they represent an almost negligible fraction of Nias' maternal gene pool.

Notably, mtDNA data do not mimic the genetic situation observed for NRY, with a strong subdivision between southern and northern Nias. This may be explainable by an exchange of women (but not men) between the exogamous patrilineal and patrilocal clans leading to a more homogeneous distribution of mtDNA rather than NRY haplogroups across the island. The only endogamous group in Nias, the *Si'ulu* in the southern part of the island, might have escaped this process, although our mtDNA data provide no strong support for this, except perhaps in the absence of haplogroup B4-16261 in this group. Considering mtDNA, Nias has an outlier position in a 3D MDS plot albeit less extreme than seen with NRY data (fig. 6). This difference may be explained by a higher effective population size in Nias for females than for males, so that the original mtDNA variation of the Nias settlers was less affected by genetic drift.

Genetic Homeland of Contemporary Nias Islanders

All NRY and virtually all mtDNA lineages we detect in our large collection of Nias islanders can be attributed to

Austronesian ancestors, who most likely originated in Taiwan and spread into Island Southeast Asia via the Philippines about 4,000–5,000 years ago (Bellwood 1997; Bellwood and Dizon 2005). Hence, our data from paternally and maternally inherited genetic markers are in line with linguistic evidence on the Austronesian origin of contemporary Nias (Brown 2001; Gray et al. 2009). In particular, our NRY/mtDNA data reject potential homelands of contemporary Nias people in India or Burma as has sometimes been proposed (Denninger 1874; Schnitger 1939). The data also show that pre-Austronesian inhabitants of Nias, whose existence is indeed supported by archaeological findings (Forestier et al. 2005), left no significant signature in neither the NRY nor the mtDNA gene pools of the present- day islanders. Given the archaeological evidence for a pre-Austronesian occupation of the island, our genetic evidence clearly indicates a population replacement rather than an admixture scenario for the human history of Nias.

Bottleneck/founder History of Nias

In Nias, we observe a strikingly reduced genetic diversity for both NRY and mtDNA (figs. 7 and 8), an effect that is more pronounced for NRY than for mtDNA. This genetic finding appears unexpected given the close geographic proximity of Nias to the rest of the Southeast Asian world, where human genetic diversities were found to be high and comparable with other human populations worldwide (Oota et al. 2002; Kayser et al. 2003; Hill et al. 2007), despite the Austronesian history in Southeast Asia being fairly recent, going back not longer than about 4,000–5,000 years ago (Bellwood 2005). Several cases are known of populations that show highly reduced genetic diversity as a result of a strong bottleneck or founder effect, in combination with geographic isolation, with the most prominent example being the Polynesians (Flint et al. 1989; Sykes et al. 1995; Hurles et al. 1998; Kayser et al. 2006). However, in contrast to Nias, reduced genetic diversity is expected for Polynesians because their settlement history involved crossings of thousands of kilometers of open sea by presumably rather small groups of people, leading to strong genetic drift effects. Furthermore, subsequent isolation applies to Polynesians because of the vast geographic distance of Polynesian islands from the assumed regions of origin in Asia and Near Oceania (Kayser et al. 2006). Both scenarios do not apply to Nias. Previous studies have shown that patrilocality, the common residence practice in Nias, can result in reduced NRY diversity within groups but increased diversity among groups (Chaix et al. 2007). However, this phenomenon cannot serve as major explanation for the case of Nias because we find a reduced Y diversity for the entire island population. Also, previous studies have demonstrated that patrilocality usually leads to reduced NRY diversity without leading to reduced mtDNA diversity (Oota et al. 2001; Kayser et al. 2003), but this was not observed in Nias where also mtDNA diversity is reduced, albeit not as drastically as NRY diversity. The reduction of population size as result of the slave trade, although expected to have affected males to a larger extent than females, is unlikely to have had a major influence on overall genetic diversity of Nias because the removal of people by slavers is suggested to have proportionally only concerned a small fraction of the total island population (Reid 2004). All our genetic evidence thus suggests an unexpected and previously undetected severe bottleneck or founder event, in combination with subsequent genetic isolation, in the human history of Nias that affected mostly men but, albeit to a lesser extent, also women, which is not explainable with current historical records. Notably, the peculiar features of the Nias gene pool as observed here for NRY and mtDNA are in line with earlier findings of blood group O being highly frequent (72.2%) in Nias and much higher than usually observed in Southeast Asia (Maasland 1939; Bijlmer 1943). Populations that underwent strong bottleneck/founder events in their history, and remained genetically isolated for a longer period of time, are usually characterized by a high degree of linkage disequilibrium in their genomes and therefore have been widely used for the mapping of disease-causing genes (Jorde et al. 2000; Peltonen et al. 2000; Shifman and Darvasi 2001; Kristiansson et al. 2008). If the reduced genetic diversity of the Nias population, demonstrated here for NRY and mtDNA, is confirmed by future autosomal DNA data, these results will call for the suitability of the Nias population for genetic association studies aiming to identify genes underlying human disease traits (Rosenberg et al. 2010). Examples of diseases worth studying include gout (OMIM:138900) (Cheng et al. 2004) and hemoglobin-associated traits, such as thalassemia (Zhang et al. 2008), sickle cell anemia (OMIM:603903) (Driss et al. 2009), and malaria susceptibility (OMIM:6111262) (Louicharoen et al. 2009), which all have a high prevalence across Southeast Asia, and might therefore be expected to exist at appreciable frequency in Nias.

Supplementary Material

Supplementary figures S1 and S2 and Supplementary tables S1 to S3 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Note Added in Proof

A very recent study (Gunnarsdóttir et al. 2010) reported three complete mtDNA sequences from the Philippines (GenBank accessions GU733719, GU733734 and GU733819) that carry the 16246T transversion that we also observed in five individuals from Nias. The three Philippine sequences clearly belong to haplogroup M74 (Zou et al. 2010). We therefore postulate that the mtDNA lineage we herein referred to as M-16246T in all likelihood can be attributed to haplogroup M74.

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