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Original Research Article

Alu Polymorphisms in the Waorani Tribe from the Ecuadorian Amazon Reflect the Effects of Isolation and Genetic Drift

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Objectives: The Amazon basin is inhabited by some of the most isolated human groups worldwide. Among them, the Waorani tribe is one of the most interesting Native American populations from the anthropological perspective. This study reports a genetic characterization of the Waorani based on autosomal genetic loci.

Methods: We analyzed 12 polymorphic *Alu* insertions in 36 Waorani individuals from different communal longhouses settled in the Yasuní National Park.

Results: The most notable finding was the strikingly reduced genetic diversity detected in the Waorani, corroborated by the existence of four monomorphic loci (ACE, APO, FXIIIB, and HS4.65), and of other four *Alu* markers that were very close to the fixation for the presence (PV92 and D1) or the absence (A25 and HS4.32) of the insertion. Furthermore, results of the centroid analysis supported the notion of the Waorani being one of the Amerindian groups less impacted by gene flow processes.

Conclusions: The prolonged isolation of the Waorani community, in conjunction with a historically low effective population size and high inbreeding levels, have resulted in the drastic reduction of their genetic diversity, because of the effects of severe genetic drift. Recurrent population bottlenecks most likely determined by certain deep-rooted sociocultural practices of the Waorani (characterized by violence, internal quarrels, and revenge killings until recent times) are likely responsible for this pattern of diversity. The findings of this study illustrate how sociocultural factors can shape the gene pool of human populations. Am. J. Hum. Biol. 23:790–795, 2011.

The western Amazon region in South America is home to a great diversity of indigenous ethnic groups, including some of the world's last uncontacted peoples that have lived in voluntary isolation (Finer et al., 2008). Among these isolated human groups, one of the most interesting from the anthropological viewpoint is the Waorani people.

The Waorani are an indigenous tribe that inhabits much of the lowland Amazon tropical forests (Wallis, 1973), with a demographic size between 1,500 and 2,000 individuals at present (Beckerman et al., 2009). The traditional Waorani territory was spread over an area of approximately 2 millions hectares, between the right bank of the Napo River and the left bank of Curaray River, in the eastern Ecuadorian provinces of Orellana, Napo, and Pastaza (see Fig. 1). This region includes the Yasuní National Park, a Pleistocene forest refugium of the Amazon basin, which together with the Waorani Ethnic Reserve delineates what the Ecuadorian government has called the Yasuní Biosphere Reserve.

The Waorani are descendants from hunter–gatherer groups, and they continue to subsist primarily on hunting and horticulture with a semi-nomadic lifestyle. While the traditional Waorani groups composed of 6–10 families practiced a nomadic lifestyle, moving regularly from one camp to another, current way of life is becoming increasingly sedentary (Rival, 2002). Regarding social organization, the Waorani society is a highly inbred and homogeneous population, according to findings from studies on the polymorphism of red cell enzymes, immunoglobulin allotypes, and dermatoglyphics (Larrick et al., 1985). This is probably the consequence of a marked predominance of bilateral cross-cousin marriages, which have been usually arranged by the parents of the young couple (Robarchek and Robarchek, 1992; Beckerman et al., 2009). They nevertheless allowed, on occasion, polygamy (polygyny and, less frequently, polyandry), if there was any demographic imbalance because of warfare or any other catastrophic event (Holt et al., 2004).

The Waorani speak the *Wao Tededo*, an unclassified and isolated language without any congener even in the Amazon region (Peeke, 1973, 1979). This language is also known as Waorani, Wao Tiriro, Huao, Auishiri, and Sabela, whereas the autonym is Huao Tededo. Wao Tededo is considered unique in its linguistic construction, with no known similarities with Zaparoan phonology or structure (Orr et al., 1991; Lu, 2001; Rival, 2002).

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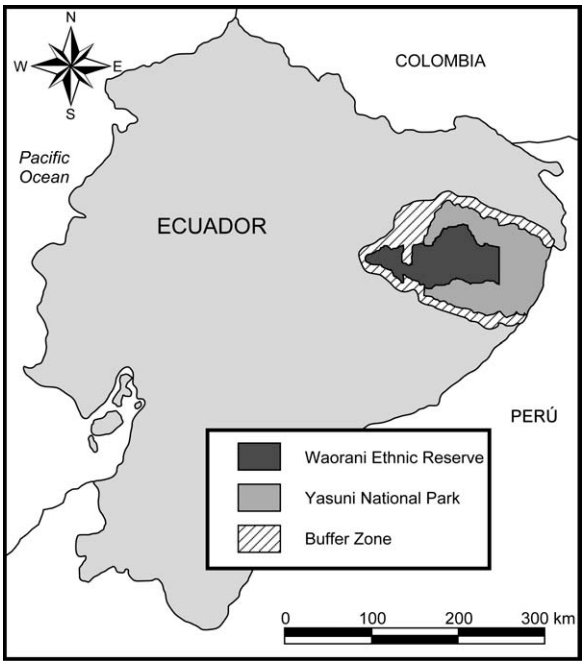

Fig. 1. Map showing the geographic location of the Waorani Ethnic Reserve, the Yasuní National Park, and the Pastaza province in Ecuador.

Settled in a violent region of the world, the western Amazon basin, the Waorani have been considered one of the most warlike societies because of their strikingly high homicide rates (Yost, 1981; Beckerman et al., 2009). Scattered over their vast territory, the Waorani fought each other almost incessantly, on account of disputes over marriage arrangements, retaliations from past killings or indictments of sorcery even among closely related groups. In a study based on extensive genealogies (up to five generations back), Yost (1981) estimated that more than a half of adult deaths (> 60%) were caused by warfare, and specifically, 44% resulted from internal quarrels. Therefore, violence and revenge have been regarded as major determinants of population mortality among the Waorani (Wallis, 1973; Robarchek and Robarchek, 1992; Boster et al., 2003; Beckerman et al., 2009).

Because of their aggressiveness, the Waorani were not colonized by other neighboring indigenous groups or by foreigners. North American missionaries made the pioneering peaceful contact of western cultures with the Waorani community in 1958 (Wallis, 1973). Subsequently, they have had limited but increasing contact with outsiders, though some Waorani groups still live completely isolated in the Amazonian forest (Yost, 1981; Boster et al., 2003).

Obviously, both the behavioral and the linguistic features of the Waorani tribe have contributed to significant population isolation, which should be mirrored in the pattern of gene diversity in this Native American population. Here, we report the genetic characterization of the Waorani tribe through the analysis of a set of polymorphic Alu insertions. These data are used to explore the effects of the isolation and a very low demographic size in the genetic diversity of the population. A sizeable number of previous publications have thoroughly discussed the advantageous properties of the *Alu* family of repetitive elements in molecular studies with evolutionary perspective. Essentially, these properties are selective neutrality of polymorphic *Alu* insertions, identity by descent not just by state, lack of insertion as the ancestral state, and no known mechanism for the complete and specific removal of an *Alu* element (Batzer and Deininger, 2002; Terreros et al., 2009; Gómez-Pérez et al., 2011, among others). All these characteristics indicate that *Alu* loci are stable and conservative markers that reflect unique evolutionary events, and hence their usefulness in the reconstruction of the human demographic and evolutionary history.

MATERIALS AND METHODS

To assess the genetic diversity of the Waorani population, we screened 12 polymorphic *Alu* insertions (A25, ACE, APO, B65, D1, FXIIIB, PV92, TPA25, HS3.23, HS4.32, HS4.59, and HS4.65). The sample consisted of 36 individuals (11 males and 25 females) living in different communal longhouses or *nanicabos* (traditional domestic groups) settled in the Yasuní National Park (Ecuador). The Yasuní National Park is a Neotropical rain forest, characterized by a warm and humid climate with an approximately constant temperature throughout the year, ranging between 18° and 24°C. We have estimated that the Waorani individuals included in the study belong to at least 12 different longhouses, bearing in mind the surname composition of the sample examined. Ethical guidelines for research with human beings were adhered to as stipulated by each of the institutions involved in the study. The Institutional Review Board from the University of the Basque Country approved the study protocol.

DNA amplification

Genomic DNA was extracted from bloodstains using the standard phenol–chloroform procedure (Sambrook et al., 1989) and stored at −20°C when not in use. PCRs were performed in a final volume of 10 μL, starting from 10 ng of DNA. Amplification conditions, annealing temperatures and primers for each *Alu* insertion can be consulted in previously published papers (Arcot et al., 1996; García-Obregón et al., 2006). PCR products were further electrophoresed in 1.5% agarose gels. The DNA bands obtained by electrophoresis were ethidium bromide-stained (0.5 μl/ml) for a better visualization and photographed under UV light. Allele identification was performed using a Roche DNA Molecular Weight Marker V ladder. Likewise, to control the quality of both the PCR and the electrophoresis, we included a positive control (homozygous for the insertion) as well as a negative control containing all the components of the PCR mix except the template DNA.

Statistical analysis

Allele frequencies with standard errors, as well as observed and expected heterozygosity for each *Alu* marker were calculated using Arlequin program v3.5 (Excoffier et al., 2010). Hardy–Weinberg Equilibrium (HWE) was tested by conducting a Fisher's exact probability test to estimate *P*-values (Guo and Thompson, 1992) with Arlequin.

To examine the genetic affinities of the Waorani with other Amerindian groups, *Alu* frequency data were compiled from the relevant literature. Populations included in

subsequent comparative analyses were Greenland Inuit, Alaskan natives, Mvskoke, Maya, Aché, Cinta Larga, Gaviao, Guarani, Kaingang, Quechua from Arequipa and from Tayacaja province in Peru, Surui, Xavante, Wai-Wai, Yanomami, and Zoró (Battilana et al. 2002, 2006). The allele frequency database was utilized to compute F_{ST} genetic distances (Reynolds et al., 1983) between all pairs of populations with the Phylip program (Felsenstein, 1989). The genetic distance matrix obtained was then represented in a two-dimensional space by applying nonmetric multidimensional scaling (MDS) analysis, using the SPSS v.16 (SPSS Inc., Chicago, IL) statistical package. Additionally, genetic heterogeneity among populations was surveyed by the exact test of population differentiation (Raymond and Rousset, 1995; Goudet et al., 1996) in the Arlequin program, to check for differences in allele frequencies between the Native American groups included in the analysis.

The relative influence of isolation, gene flow, and genetic drift on the gene pool of each of the Amerindian populations considered was explored by plotting the expected Hardy–Weinberg proportions of heterozygotes of each sample against the distance from the centroid, according to the methodology devised by Harpending and Ward (1982). Taking the potential effects of genetic drift into consideration, a strong negative relationship is expected between population divergence and heterozygosity. Consequently, it is assumed that populations plotting above the theoretical prediction given by the regression line have experienced a higher than average gene flow level, whereas those that fall below the regression line have experienced limited gene flow.

RESULTS

Allele frequencies for the *Alu* insertions typed in the Waorani sample are listed in Table 1. Three *Alu* elements (ACE, APO and FXIIIB) were monomorphic for the presence of the insertion, while HS4.65 was monomorphic for the lack of the insertion. The frequencies of the remaining *Alu* loci were distributed in a wide range (0.074–0.968). Among them, four Alu markers were close to the fixation for the presence (PV92 and D1) or the absence (A25 and HS4.32) of the insertion. Accordingly, average values for the observed (Ho: 0.207) and expected (He: 0.209) heterozygosity proved to be relatively low. Hardy–Weinberg equilibrium (HWE) was assessed by an exact test to calculate the *P*-value using the Markov-chain Monte Carlo method (Guo and Thompson, 1992). No significant departure from HWE expectations was detected in most of the eight unfixed *Alu* insertions, excepting PV92 ($P = 0.017$). After applying Bonferroni correction for multiple testing, the significance threshold (*P*-value) proved to be 0.00625, so that PV92 also conformed to HWE expectations.

Results of the exact test of population differentiation show a notable genetic heterogeneity between the Waorani and the rest of Native American samples included in the comparative analysis (see Supporting Information Table S1). The greatest number of significant differences was observed between the Waorani and the North American collections notably Mayan and Greenland Inuit, with dissimilar Alu frequencies for 7 and 8 loci (out of 12 markers). On the other hand, South American populations showed the lowest number of significantly different *Alu* frequencies relative to the Waorani sample, specifically the Zoró and Surui groups.

Results of nonmetric MDS applied to the F_{ST} genetic distance matrix to evaluate genetic relationships among populations are depicted in Figure 2. Given the paucity of allele frequency data for B65 and D1 loci in Native American populations, these Alu markers were not considered in subsequent analyses. The two-dimensional representation of the F_{ST} distance matrix accounted for 89.4% of the total variance, with a coefficient of stress of 15.2%. MDS plot roughly reflected the geographic distribution of the Amerindian groups in subcontinents. In this way, North American populations (Greenland Inuit, Alaskan natives, and Mvskoke) appeared in a clearly differentiated cluster at the negative end of dimension I. Another substantial part of the genetic heterogeneity detected in the MDS plot was introduced by some South American populations, such as Aché, Wai-Wai, Gaviao, and Yanomami, as well as Waorani. Such Amerindian groups appeared in a peripheral position, somewhat separated from the rest, probably on account of their genetic distinctiveness. Finally, the main cluster was formed by the Mayan and most of the South American collections, which plotted close to the centroid of the distribution or in the positive semiaxis of dimension 1.

The amount of gene flow experienced by the Waorani was examined by the centroid method (Fig. 3). In the resultant figure, the regression line represents the expected heterozygosity, so that populations plotting above the line would have received a higher-than-average gene flow, whereas those positioned under the regression line would have been less impacted by gene flow processes and, therefore, would have evolved in a relatively isolated milieu. As expected, the Waorani population plotted far below the regression line, indicating that the gene flow experienced by this indigenous community was substantially lower than the average computed for the whole set of Native American populations included in this work. Coinciding with MDS results, other populations that showed gene flow levels below the average proved to be the Aché, Wai-Wai, Gaviao, and the Yanomami. In con-

TABLE 1. Allele frequencies with standard errors (± SE), observed heterozygosity (Ho) and expected heterozygosity (He) for 12 polymorphic Alu insertions in Waorani population (Ecuadorian Amazon)

Locus	ACE	TPA25	APO	PV92	FXIIIB	D1	A25	B65	HS3.23	HS4.32	HS4.59	HS4.65
Freq[a]	1.000	0.500	1.000	0.968	1.000	0.900	0.074	0.414	0.552	0.107	0.652	0.000
S.E.	0.000	0.045	0.000	0.031	0.000	0.041	0.028	0.060	0.052	0.035	0.057	0.000
Ho	0.000	0.667	0.000	0.000	0.000	0.133	0.147	0.371	0.552	0.214	0.394	0.000
He	0.000	0.500	0.000	0.062	0.000	0.180	0.136	0.485	0.495	0.191	0.454	0.000
P[b]	/	0.093	/	0.017	/	0.242	1.000	0.173	0.268	1.000	0.457	/

[a]Estimated for the presence of the *Alu* insertion.
[b]*P*-values for the Fisher's exact test (Hardy–Weinberg equilibrium).

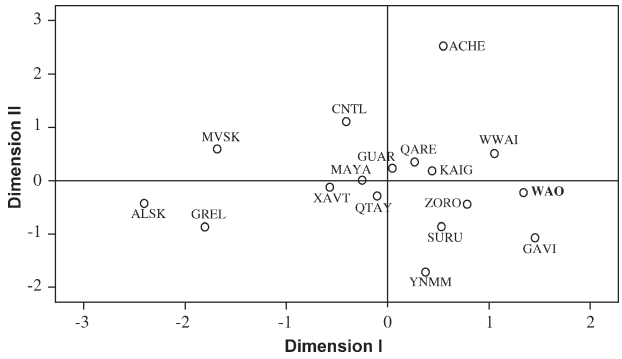

Fig. 2. Nonmetric multidimensional scaling (MDS) plot applied on a Reynolds F_{ST} genetic distance matrix for 17 Amerindian populations. Genetic distances were computed based on insertion frequencies for 10 *Alu* markers (A25, ACE, APO, FXIIIB, HS3.23, HS4.32, HS4.59, HS4.65, PV92, and TPA25). Populations key: Waorani (WAO), Mvskoke (MVSK), Alaskan Inuits (ALSK), Greenland Inuits (GREL), Maya from Yucatan (MAYA), Yanomami from Venezuela (YNMM), Quechua from Arequipa (QARE) and from Tayacaja (QTAY) provinces in Peru, Aché from Paraguay (ACHE), and Cinta Larga (CNTL), Gavião (GAVI), Surui (SURU), Wai-Wai (WWAI), Zoró (ZORO), Guarani (GUAR), Kaingang (KAIG), and Xavante (XAVT) from Brazil.

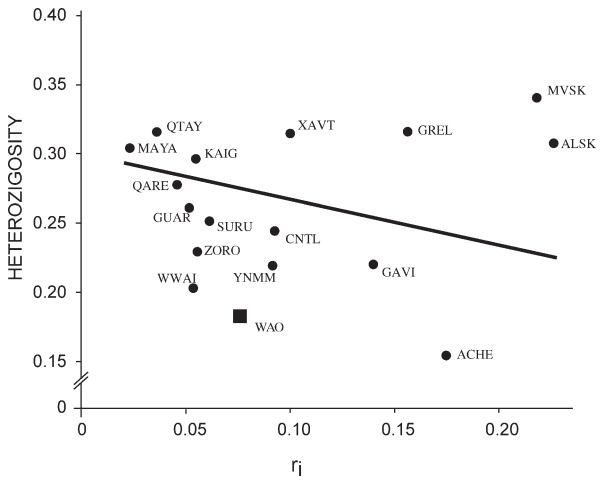

Fig. 3. Plot of heterozygosity versus distance from genetic centroid for 17 Native American populations. The line represents the expected relationship predicted by the model of Harpending and Ward (1982). Population key: Waorani (WAO), Mvskoke (MVSK), Alaskan Inuits (ALSK), Greenland Inuits (GREL), Maya from Yucatan (MAYA), Yanomami from Venezuela (YNMM), Quechua from Arequipa (QARE) and from Tayacaja (QTAY) provinces in Peru, Aché from Paraguay (ACHE), and Cinta Larga (CNTL), Gavião (GAVI), Surui (SURU), Wai-Wai (WWAI), Zoró (ZORO), Guarani (GUAR), Kaingang (KAIG), and Xavante (XAVT) from Brazil.

trast, gene flow levels above the expected ones were observed in the populations from the "northern cluster", i.e., Mvskoke, Alaskan natives, and Greenland Inuit.

DISCUSSION

This work provides new data on the genetic background of the Waorani, one of the most isolated and anthropologically interesting Native American populations, from the perspective of classical polymorphic *Alu* insertions.

Paternal (Y-chromosome) and maternal (mtDNA) lineages have been widely analyzed in human evolutionary studies to characterize human groups in terms of origin, demographic landmarks (founder effects, population bottlenecks), and genetic variability. Yet, the reconstruction of the history of human populations from genetic data is a multifaceted task that also needs information from the recombining parts of the nuclear DNA, that is, the autosomes (Kidd et al., 2000). Polymorphic *Alu* markers constitute an essential source of nuclear genetic diversity. *Alu* repetitive elements are highly stable markers that are not affected by substantial mutation rates. Therefore, *Alu* repeats form a rich molecular fossil record that is faithfully recorded in the human genome from generation to generation, which constitutes an invaluable feature in studies with evolutionary perspectives. Furthermore, *Alu* elements are widely dispersed throughout the human genome, subject to very limited amounts of gene conversion, and selectively neutral (Batzer et al. 1996). In terms of selection, most young *Alu* elements can be considered as neutral residents of the human genome (Cordaux et al. 2006). With mutation and selection events ruled out, analysis of *Alu* insertions should exclusively reflect past processes of interaction between gene flow and genetic drift.

The most remarkable finding of this study is the strikingly low genetic diversity detected in the Waorani population. The substantial loss of genetic diversity might have been caused by intense genetic drift episodes associated with both founder effects during the early settlement of the New World (Novick et al., 1998), and with subsequent recurrent population bottlenecks probably conditioned by some behavioral peculiarities of this Amerindian community. Until very recently, the Waorani of eastern Ecuador engaged in an endless cycle of revenge killing in which men responded to the death of kin by attacking their enemies (Boster et al., 2003). This sociocultural scenario was predominantly characterized by inevitable conflicts, anger and hostility, so that an important fraction of the population mortality could be ascribed to internal fights, violence and warfare episodes (Yost, 1981). Mortality crises resulting from quarrels within the tribe would have triggered strong population bottlenecks, thus promoting recurrent genetic drift events.

Along these lines, some authors have suggested that the Waorani population size in the 1950s did not exceed 500 individuals (Yost, 1981; Holt et al., 2004), which dispersed over $\sim$ 20,000 km^2 renders an extremely low population density (0.025 inhabitant/km^2). Likewise, the demographic size of the Waorani community toward 1993 was estimated in around 1300 individuals (Smith, 1993). Nowadays, population density remains low at $<$1 inhabitant/km^2 (Beckerman et al., 2009). In such a context, the impact of the genetic drift might have been reinforced by: i) a marked population isolation, and therefore, a virtually nonexistent effect of the gene flow as a consequence of the behavioral and linguistic singularities of the Waorani, and their reluctance to any contact with other human groups, and ii) a historically low effective population size, on account of high mortality rates and low reproductive performances (Beckerman et al., 2009).

As we have mentioned, the main consequence of population bottlenecks and the genetic drift associated would have been a drastic decline of the Waorani's genomic diversity. In this work, the reduction of the genetic diversity in the Waorani was corroborated by the substantial allelic

loss of the *Alu* loci examined: four (ACE, APO, HS4.65, and FXIIIB) out of 12 *Alu* markers proved to be monomorphic, whereas other four *Alu* elements were very close to the fixation for the presence (D1, PV92) or the lack (A25, HS4.32) of the insertion. The markedly low heterozygosity of this Amerindian population is in all probability strengthened by the peculiar marital structure of the Waorani, with wide predominance of close consanguineous matings (Larrick et al., 1985).

In a study on the Asian origin of native American groups based on five polymorphic Alu insertions, Novick et al. (1998) found six out of 24 Central and South American populations featuring very low genetic diversity, with heterozygosity values below 0.20. Specifically, these populations were the Ngobe from Western Panama (0.19 ± 0.08), Arhuaco (0.17 ± 0.06) and Chimila (0.18 ± 0.10) from Northern Colombia, Guayabero (0.15 ± 0.09) from East-central Colombia, and Karitiana (0.16 ± 0.09) and Surui (0.19 ± 0.07) from the Brazilian Amazon. The cited authors pointed out that, bearing in mind that Aborigines from the New World are thought to derive from migrant groups with a limited number of individuals, genetic diversity is expected to be reduced in some Amerindian populations because of founder effects and population bottlenecks. The heterozygosity obtained for the same five Alu insertions (ACE, TPA25, APO, PV92, and FXIIIB) was even lower in the Waorani collection (0.13 ± 0.07). This result corroborates the low diversity of the study population and seems to support the idea that genetic drift events other than those generated by the early settlement of the Americas could have had an impact on the loss of genetic diversity in the Waorani.

The isolated condition of the Waorani tribe is clearly perceptible in the results of the centroid analysis (see Fig. 3). Their remote position below the regression line (i.e., below the expected heterozygosity) gives credence to the notion of the Waorani being one of the Native American groups less impacted by gene flow processes. In contrast, the Mvskoke, which have been reported to have experienced a substantial degree of admixture with non-American native groups (Kasprisin et al., 1987; Novick et al., 1998), appeared with gene flow levels far above the average.

Previous analyses of the Waorani population based on Y-chromosome microsatellites and mitochondrial DNA have also found a significantly low genetic diversity for this interesting anthropological group. Results derived from the analysis of Y-chromosomal short tandem repeats (Y-STRs) revealed reduced haplotype variability within the population, and a large genetic distance between the Waorani and the major Ecuadorian ethnic groups (González-Andrade et al., 2009). Similarly, studies of the mitochondrial DNA D-loop region revealed the significant homogeneity of Waorani maternal lineages, with a wide predominance of a Waorani-specific variant of the haplogroup A2 (Cardoso et al., 2008; Baeta et al., 2009).

Other Native American samples such as Ache, Yanomami, Wai Wai, and Gaviao plotted considerably below the expected heterozygosity values as well, in accordance with previously published data (Battilana et al., 2006). Among them, the Yanomami have been reported to possess quite similar habitat conditions and behavioral characteristics than those of the Waorani, including aggressiveness (Chagnon, 1968, 1988). Nevertheless, some peculiarities regarding the cultural rules that guide these two hostile lowland South American peoples have led the specialists to consider the Waorani even more warlike than the Yanomami (see Beckerman et al., 2009 for a review). One major difference between the two societies is the spacing of revenge raids in the Yanomami. Sociocultural mores that guide the Yanomami violence dictate that an interval of respite follows an exchange of homicides, during which both sides usually stand down for about a generation. On the other hand, there is no evidence that the Waorani have any type of cultural regulation mandating a lapse of time (or limitation in the number of victims) in revenge exchanges. In other words, no tradition of standing down (even for a short period) has been observed in the Waorani.

Contrasting cultural patterns may also be involved in another crucial difference between the cited societies. Among the Yanomami, male deaths from warfare and/or homicide have been reported to be 5–10 times more numerous than female deaths. In contrast, among the Waorani, male deaths (at all ages) caused by internal quarrels and revenge killings have been reported to be only 1.4 times more numerous than female deaths from the same cause. This peculiar behavior of killing women and girls might be a key factor in explaining the historical failure of the population growth of Waorani community. Likewise, this demographic contrast between Yanomami and Waorani may account for differences in the intensity of population bottlenecks, and therefore, in the differential impact of genetic drift on the genetic diversity of these two Native American groups.

The prolonged isolation of the Waorani, along with a historically low effective population size (Yost, 1981; Boster et al., 2003; Beckerman et al., 2009) and high endogamy levels (Larrick et al., 1985), have resulted in the drastic reduction of their genetic diversity, because of the effects of strong genetic drift episodes. Recurrent population bottlenecks presumably determined by certain deep-rooted sociocultural practices of the Waorani (characterized by violence, internal quarrels and revenge killings until recent times) are likely responsible for their genetic singularity, and therefore, for their genetic microdifferentiation with respect to other Native American populations. In summary, the findings of this study illustrate the importance of sociocultural factors in the variation and evolutionary history of the human gene pool.

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