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RESEARCH ARTICLE

# Genetic diversity at the edge: comparative assessment of Y-chromosome and autosomal diversity in eastern chimpanzees (*Pan troglodytes schweinfurthii*) of Ugalla, Tanzania

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**Abstract** One of the three categories of biodiversity for conservation priority recommended by the International Union for the Conservation of Nature is genetic diversity. In this study, we estimate the genetic diversity of eastern chimpanzees in the Ugalla region of western Tanzania, which represents the easternmost distribution of the subspecies *Pan troglodytes schweinfurthii*. We collected 237 fecal samples from a 624 $km^2$ area of the Ugalla region, analyzed the DNA at 12 autosomal loci and identified 113 individuals (69 males and 44 females). We also analyzed 13 Y-chromosome loci in the Ugalla males. While autosomal genetic diversity is within the range of other eastern populations, at 0.27 the gene diversity of the Y-chromosome haplotypes present among 61 Ugalla males is extremely low as compared to other eastern chimpanzee populations. In addition, the most prevalent haplotype, found in 52 of the males, is distributed across the entire surveyed area of 624 $km^2$. This low level of paternally-transmitted genetic diversity among the Ugalla males may be the result of a small or highly related, recent founder population (i.e., genetic drift), exacerbated by the male philopatric structure of chimpanzee communities and by male reproductive skew.

**Keywords** Genetic diversity · Y-chromosome · Chimpanzees · Heterozygosity · Male philopatry

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## Introduction

Great ape populations are in serious decline (Caldecott and Miles 2005; Walsh et al. 2003; Johnson et al. 2005; Campbell et al. 2008), and methods to quantify the viability and demographic stability of current populations urgently are needed to inform conservation science. There are many ways to assess the viability of a population. For example, nest surveys are conducted to estimate the number of individuals left in the wild and to determine their geographic distribution (Pruetz et al. 2002; Moore 1985; Johnson et al. 2005; Kormos et al. 2003; Moyer et al. 2006). At long-term field study sites, effective population size (*Ne*) and life tables are calculated to understand demographic structure (Boesch and Boesch-Achermann 2000; Goodall 1986). The extent of the remaining great ape habitat also is measured through remote and on-the-ground surveys (Devos et al. 2008; Ancrenaz et al. 2005; Junker et al. 2012; Pintea et al. 2002). As important to the survival of great apes in the wild as these metrics are, so too is the maintenance of genetic diversity among primate populations.

Genetic diversity is an important measure in conservation science and is one of three biodiversity levels recommended for conservation by the International Union for the Conservation of Nature (IUCN). Most often measured as heterozygosity (i.e., the proportion of loci that have two different alleles in the average individual), a high level of genetic diversity is expected to provide adequate variation for a species to evolve in response to environmental challenges in the long term, and in the short term, increase fitness through the avoidance of inbreeding depression (Reed and Frankham 2003; Freeland 2005). In a meta-analysis designed to measure the connection between heterozygosity and measures of fitness, Reed and Frankham (2003) found that levels of genetic variation may explain

19 % of the variation in fitness across 21 species investigated.

One of the most important contributors to genetic variation is gene flow (Freeland 2005). The introduction of novel genetic material into a population through the immigration of individuals is thought to be necessary for the persistence of a population (Wright 1949). As great ape habitats become more fragmented across Africa and Asia, the movements of individuals between isolated populations is restricted, eventually leading to increases in inbreeding and decreases in heterozygosity. This threat to genetic diversity may be exacerbated by intrinsic factors of great ape social structure and organization, such as sex-biased dispersal, long generation lengths, and reproductive skew (Wang 2004). Chimpanzees at all study sites live in male philopatric communities, wherein the males—and their genetic material—almost always remain in their natal group, while the females migrate to other communities at adolescence. In addition to this restriction of Y-chromosome gene flow, chimpanzee males live in a strict hierarchy, in which the alpha male sires more offspring than any other individual male during his tenure, often resulting in a pronounced short term reproductive skew (Boesch et al. 2006; Constable et al. 2001; Newton-Fisher et al. 2010; Vigilant et al. 2001; Wroblewski et al. 2009; Langergraber et al. 2013). Given that it is haploid (i.e., half the number of autosomal chromosomes) and male philopatric social structure restricts the flow of Y-chromosomes between communities, Y-chromosome diversity is therefore at a high risk of decrease within chimpanzee communities, and thus may reflect the effects of a small or isolated population more quickly than other parts of the genome (van Oven et al. 2011).

While male-mediated gene flow is highly restricted due to male philopatry, Y-chromosomes can be dispersed via extra-group paternity (Vigilant et al. 2001; Newton-Fisher et al. 2010; Schubert et al. 2011), and perhaps due to secondary transfer of parous females with male offspring (Nishida 1985; Boesch et al. 2008; Emery Thompson et al. 2006). Another way that chimpanzee communities may share Y-chromosome haplotypes is through common ancestry. Chimpanzees live in flexible fission–fusion communities, wherein community members fission into smaller parties or fusion to create larger parties, depending on several factors such as food availability, number of estrous females, threat of predation, and demographic factors (Lehmann and Boesch 2004; Conklin-Brittain et al. 1998; Matsumoto-Oda 1999; Pruetz et al. 2007). If community size increases to the point that available resources are insufficient for all individuals, or conversely as at Gombe, when a highly defendable resource became available (i.e., provisioned bananas) (Goodall 1986), a community fission may become permanent, resulting in a new community. The male members of the new community would carry a subset of the Y-chromosome haplotypes of the community they fissioned from, and these haplotypes are likely to persist in some males, even as new mutations occur. Thus, Y-chromosome haplotypes shared between communities are likely 'ancestral,' in that they are likely to have occurred in a past, 'founder' community.

Genetic diversity may also be lost as populations expand, due to population bottlenecks or founder effects, wherein the individuals at the leading edge of a species' distribution carry only a subset of the genetic variation found at the center (Hewitt 2004), as observed in recent studies (Arnaud-Haond et al. 2006; Böhme et al. 2007; Diekmann and Serrão 2012; Rowe et al. 1999). These edge populations often live in habitats that represent the extreme environmental limits of their adaptability, such as the population of chimpanzees living in the savanna-woodlands of Ugalla in western Tanzania. Edge populations may also carry novel mutations, as natural selection acts on existing variation to engender specific adaptations to an extreme environment, and therefore may represent important populations for the persistence of a species (Lesica and Allendorf 1995).

The eastern chimpanzees (*P. troglodytes schweinfurthii*) (Schwarz 1934) of Ugalla represent the easternmost expansion of this subspecies' range into a less species-typical savanna-woodland habitat (Fig. 1). Eastern chimpanzees are one of four subspecies of chimpanzee, and are the most recently diverged, having split from the central subspecies (*Pan troglodytes troglodytes*) approximately 77,000–250,000 years ago (Stone et al. 2010; Gonder et al. 2011; Hey 2010; Bjork et al. 2011). Studies of the demographic history of the chimpanzee subspecies have investigated the relative levels of genetic variation within three of the subspecies; however, results vary according to which part of the genome is measured. Autosomal genetic variation was found to be lowest in the western subspecies (*Pan troglodytes verus*), followed by the eastern, then the central subspecies (Becquet et al. 2007; Fischer et al. 2006). These findings are consistent with the proposition that central chimpanzees represent the ancestral population, from which the eastern and western subspecies diverged, and the relatively low genetic diversity among the western subspecies may be due to a small effective population size since divergence (Becquet et al. 2007). In contrast, the western subspecies demonstrated the highest levels of diversity in mitochondrial DNA (mtDNA), perhaps due to a female-skewed founder population (Stone et al. 2010), while the eastern subspecies had very low mtDNA diversity, possibly explained by a recent restriction in population size (i.e., 'bottleneck') (Goldberg and Ruvolo 1997). Discordance between measures of autosomal and sex-linked DNA diversity is most often attributed to the

divergent behavior of males and females within a species, such as sex-biased dispersal and male reproductive skew, and with the lower effective population size of haploid relative to diploid chromosomal loci (Hammond et al. 2006; Eriksson et al. 2006; Segurel et al. 2008). The pattern of genetic variation observed in eastern chimpanzees suggests that this subspecies may have undergone a population bottleneck, followed by a more recent expansion in numbers (Gagneux et al. 1999; Fischer et al. 2006).

The chimpanzees of Ugalla represent the final expansion of this Endangered (IUCN 2012) species across Africa, and it is therefore important to assess the genetic diversity of this population living at the distribution edge, and in an extreme environment for the species. In addition to their tenuous position at the leading edge, the Ugalla chimpanzees may also experience restricted gene flow. The Mpanda-Uvinza road separates Ugalla from the neighboring Masito region, and may be the only migration route available. Investigation of mtDNA and SIV antibodies have provided evidence, however, that the Ugalla chimpanzees have exchanged genetic material with neighboring populations, at least in the past. This study describes the Y-chromosome and autosomal genetic diversity present in a sample of the Ugalla population and compares this diversity to that found in other chimpanzee populations.

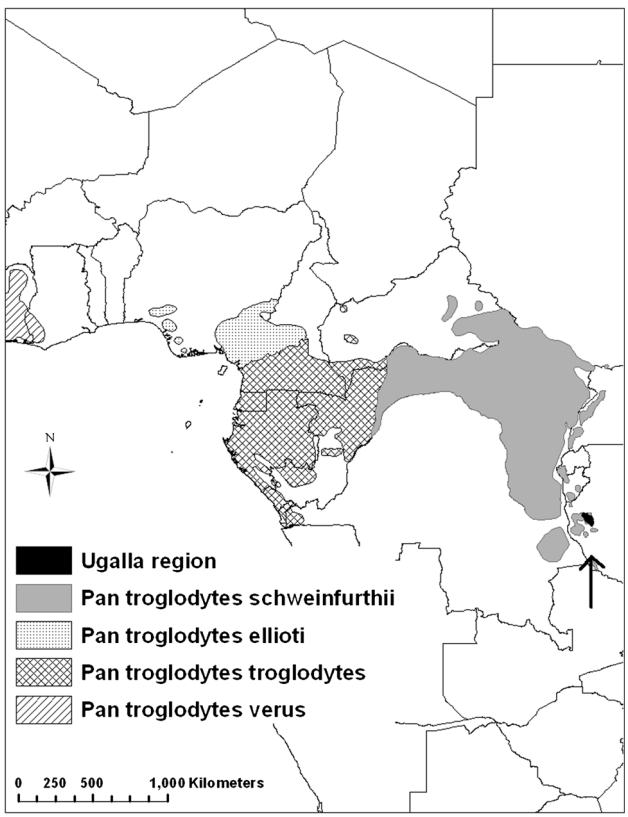


**Fig. 1** Distribution of the four subspecies of chimpanzee across equatorial Africa, and the location of the Ugalla study site

## Methods

### Study site and subjects

The Ugalla region is located approximately 80 km west of Lake Tanganyika on the plateau of western Tanzania (Fig. 2). It covers approximately 3,350 km$^2$ and is characterized as a savanna-woodland, dominated by the tree genera *Julbernardia* and *Brachystegia* that comprise what is known as 'miombo woodland' (Moore 1994). The region receives less than 1,000 mm of rain per year and experiences an extended drought between May and October (Hernandez-Aguilar 2006). The area surveyed for this study ranges in elevation from 1,095 to 1,919 m above sea level. Ugalla is part of the Greater Mahale Ecosystem (Fig. 2), which contains almost the entire free-ranging population of Tanzania's chimpanzees (Plumptre et al. 2010). As stated above, there are no chimpanzees east of Ugalla, and the area is bounded on the southwest by the Uvinza-Mpanda road, and by the Malagarasi River to the north (Kano 1972) (Fig. 2).

*Pan troglodytes schweinfurthii* (Oates et al. 2009; Schwarz 1934) range from the Democratic Republic of Congo (DRC) and the Central African Republic (CAR) in the west, through Uganda, Burundi, Rwanda, and Tanzania to the east (Fig. 1). The chimpanzees in Ugalla are not habituated to human presence and are not located in a national park, although part of the region falls within the Tongwe East Forest Reserve.

Data from other chimpanzee populations included in this study were taken from publications. Eastern chimpanzee autosomal data came from the Budongo Forest Reserve (Sonso community) in Uganda (Lukas et al. 2005), the Gishwati Forest Reserve in Rwanda (Chancellor et al. 2012), and the Gombe Stream National Park (Kasakela community) in Tanzania (Constable et al. 2001). Y-chromosome haplotype data came from three study sites in Uganda: the Budongo Forest Reserve (Sonso community), the Kibale Forest National Park (Ngogo and Kanyawara communities) (Langergraber et al. 2007), and Semliki National Park (Mugiri community) (Langergraber et al. 2007); and from the Gishwati Forest Reserve in Rwanda (Chancellor et al. 2012). Autosomal diversity results also were compared to a population of western chimpanzees in the Taï National Park, Ivory Coast (North, Middle, and South communities) (Lukas et al. 2005), as this subspecies (i.e., *P. troglodytes verus*) has been reported to carry the lowest levels of nuclear genetic diversity.

This study complied with protocols approved by the Institutional Animal Care and Use Committee at the University of Texas at San Antonio (#PA001-05/14A0).

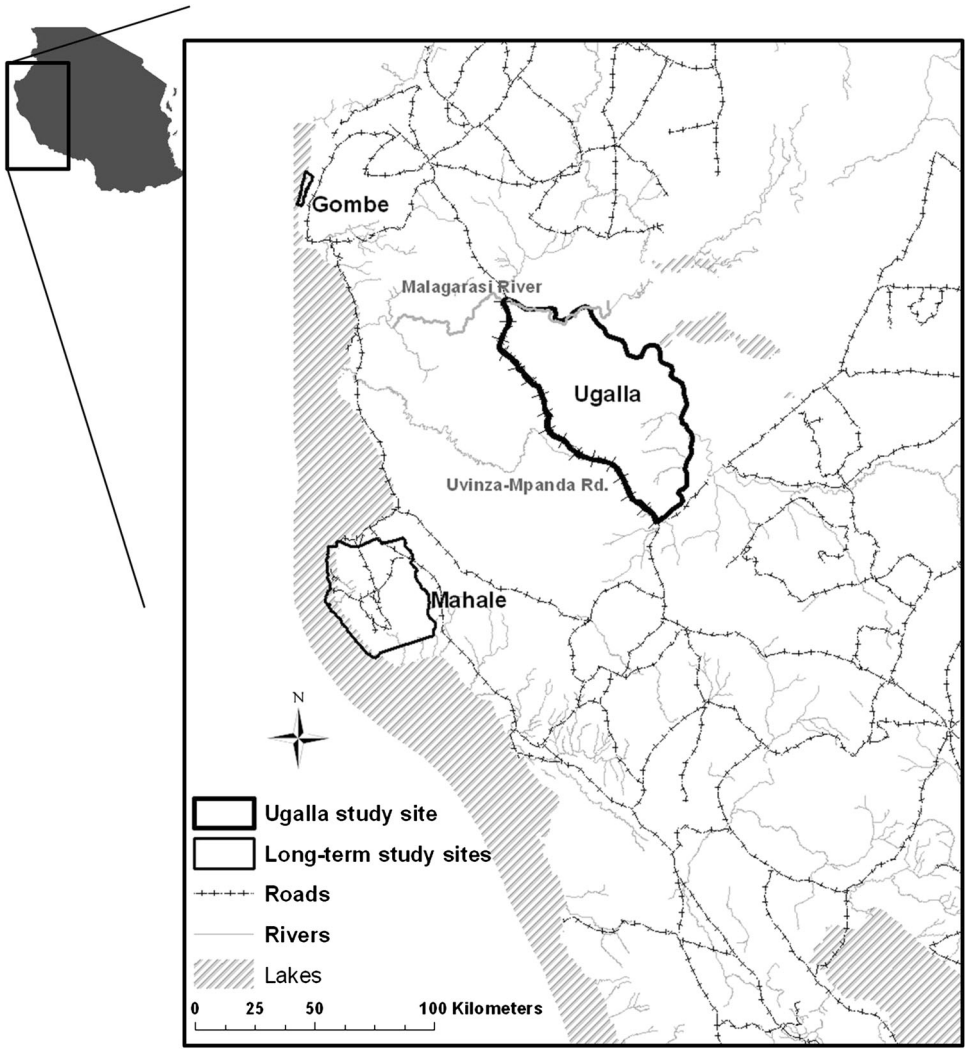


**Fig. 2** Location of Ugalla in Tanzania and in reference to long-term chimpanzee study sites, and demonstrating the boundaries of the Malagarasi River and the Uvinza-Mpanda Road. Road and river data downloaded from GeoComm International Corp (2011)

## Sample collection

Genetic samples, which included feces, food wadges (the discarded bolus of chewed seeds/leaves/fruit pulp left once the juice has been sucked out of it), and a sperm plug, were non-invasively collected, between July 2009 and April 2010, across a 624 km$^2$ portion of the Ugalla region (Fig. 3). Surveys to collect samples were conducted from one permanent research camp and from five temporary camps that were established across a predetermined grid, to best sample the study area (Fig. 3). The survey area was determined based on previous nest surveys, remote sensing, and accessibility from the main road, and was large enough to ensure a representative sampling of genotypes from the area and that several chimpanzee communities would be sampled. The duration at each camp was 15–33 days. From each camp, surveys were biased towards following any sighted chimpanzees, and strict transects were not walked; however, the entire area within a minimum 4 km radius was searched from each camp, based on the predetermined grid (Fig. 3).

Samples were collected using the two-step ethanol-silica preservation method (Nsubuga et al. 2004) and were stored in the field for upto 5 months. Samples were shipped from Kigoma, Tanzania, to the Max Planck Institute for Evolutionary Anthropology (MPI-EVA) in Leipzig, Germany, where they were stored for up to 18 months at 4 °C.

## DNA extraction and amplification

All extractions were conducted in the Department of Primatology at MPI-EVA, using the QIAmp DNA stool kit (Qiagen) and according to manufacturer's instructions and amendments as per Nsubuga et al. (2004). Extracts were amplified in four independent reactions at the X–Y homologous amelogenin locus and electrophoresed on an agarose gel to assess presence of DNA, and for later determination of sex (Bradley et al. 2000). Based on these results, extracts with DNA products were further amplified in a two-step multiplex procedure (Arandjelovic et al. 2009). The extracts were first amplified at 19 autosomal microsatellite loci, using

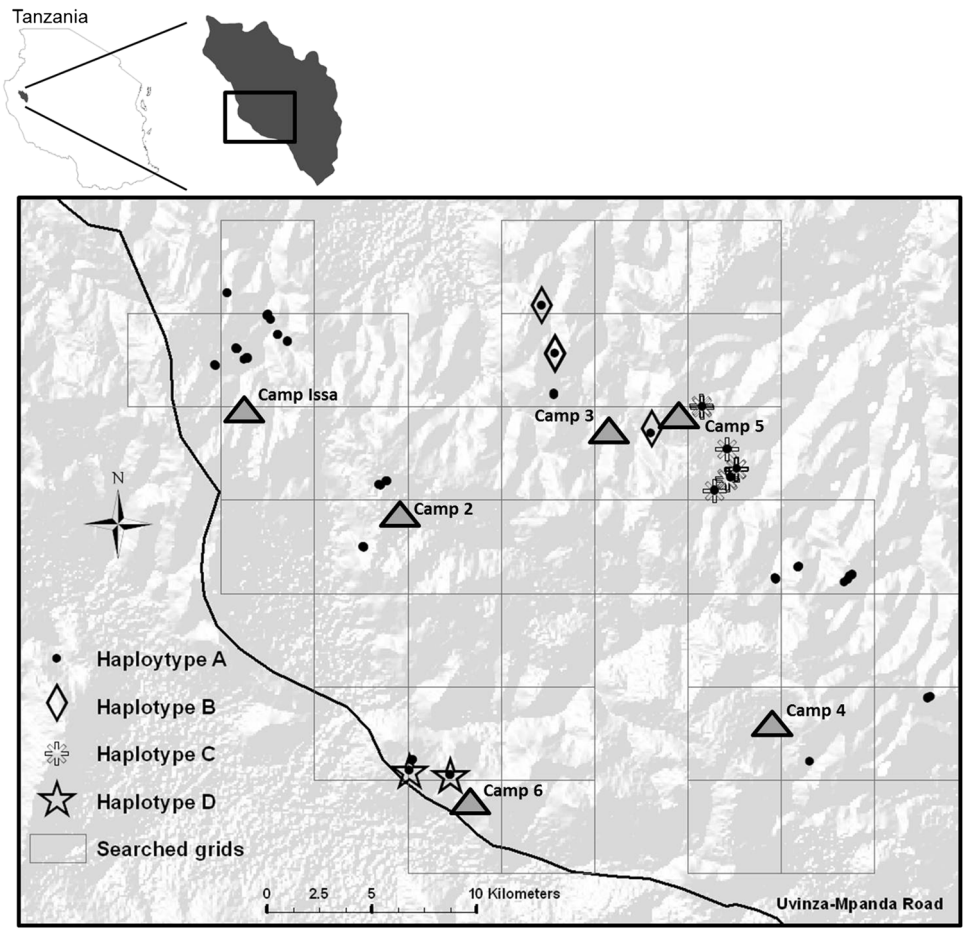


**Fig. 3** Distribution of the Y-chromosome haplotypes in the surveyed area of the Ugalla region, demonstrating the presence of the predominant haplotype A throughout the study area, and including the location of each camp. Topographic data downloaded from GeoComm International Corp (2011)

unlabeled primers, and then amplified at a subset of 12 loci using fluorescently-labeled forward primers and nested reverse primers (Table 1) (Arandjelovic et al. 2009). The PCRs were conducted as detailed in Arandjelovic et al. (2009), with the exception of the second singleplex step, wherein the reagent volume was halved to 10 µl.

In previous studies (e.g., Arandjelovic et al. 2010), a minimum of eight loci were necessary to distinguish the genotypes of individuals, and so 12 loci were analyzed here to ensure that our data would distinguish individuals in our study. The 12 microsatellite loci were chosen based on successful amplification in a previous study of eastern chimpanzees (Langergraber et al. 2007). Depending on amplification success, between three and 12 independent amplifications were performed for each extract at these 12 loci (Arandjelovic et al. 2009). Genotypes were confirmed heterozygotes when each allele was observed in two or more independent reactions, and homozygotes were confirmed when the single allele was observed in three to five independent reactions, depending on the amount of template DNA in the sample (Arandjelovic et al. 2009).

The sex of the individual represented by each extract was determined from the amplification of a portion of the XY homologous amelogenin locus, wherein heterozygotes were considered male and homozygotes female (Bradley et al. 2001). To distinguish individuals, we used the software *Cervus version* 3.0 (Kalinowski et al. 2007) to first calculate allele frequencies of each locus amplified for the Ugalla population; based on these frequencies, the number of loci necessary to confidently ($P_{IDsibs} \leq 0.001$) assign consensus identifications to extracts with the same genotype was then determined. Extracts with genotypes that mismatched at one or two loci were examined for genotyping errors, and re-amplified in cases of ambiguity.

Once individual identifications and sex were determined, we amplified one extract from each male at 13 Y-chromosome loci (DyS392, DyS439, DyS469, DyS502, DyS510, DyS517, DyS520, DyS533, DyS562, DyS588, DyS612, DyS630, DyS632) (see Langergraber et al. 2007 for primer sequences and reaction conditions). The same two-step multiplex procedure was used and all 13 loci were amplified in the second step single locus PCR reactions.

## Genetic diversity

### *Autosomal*

For the 12 autosomal microsatellite loci amplified, several measures were calculated using the software *Arlequin*

**Table 1** Microsatellite loci amplified for each for the sites compared in this study, with the coincident subset in bold

| Ugalla | Gishwati | Sonso | Taï | Gombe |
|---|---|---|---|---|
| D11s2002 | D11s2002 | | | |
| **D2s1326** | **D2s1326** | **D2s1326** | **D2s1326** | D2s1326 |
| **D7s817** | **D7s817** | **D7s817** | **D7s817** | |
| **D5s1470** | **D5s1470** | **D5s1470** | **D5s1470** | |
| D6s1056 | D6s1056 | | | |
| D5s1457 | D5s1457 | | | |
| D1s1622 | D1s1622 | | | |
| **D2s1329** | **D2s1329** | **D2s1329** | **D2s1329** | |
| D3s2459 | | | | |
| D7s2204 | | D7s2204 | D7s2204 | |
| D14s306 | | | | |
| D16s2624 | | | | |
| | D4s1627 | | | D4s1627 |
| | D1s1656 | | | |
| | D10s676 | | | D10s676 |
| | D3s3038 | | | |
| | | D9s910 | D9s910 | |
| | | D11s200 | D11s200 | |
| | | 2D12s66 | 2D12s66 | |
| | | vwf | vwf | |
| | | | | D2s1333 |
| | | | | D4s243 |
| | | | | D1s548 |
| | | | | D9s922 |
| | | | | D11s1366 |
| | | | | D2s433 |
| | | | | HUMFABP |
| | | | | D20s470 |
| | | | | D9s302 |
| | | | | D18s851 |
| | | | | D19s431 |
| | | | | D9s905 |
| | | | | D18s536 |

*version* 3.5 (Excoffier et al. 2005). For diploid data, the most commonly used measure of genetic diversity is expected heterozygosity ($H_e$), which is the probability that any two alleles at a single locus, chosen at random from the sampled population, are different from each other, based on the allele frequencies at each locus. When averaged over all 12 microsatellite loci used in this study, an estimate of the extent of genetic variability in the Ugalla population was determined (Nei 1973, 1978). Expected heterozygosity was compared to the observed heterozygosity ($H_o$) to determine deviation from Hardy–Weinberg equilibrium ($F_{is}$) (Nei 1977). An important corollary to genetic diversity, or expected heterozygosity, is the effective number of alleles ($A_e$), given that populations may have the same number of alleles per locus, but have very different levels of genetic diversity (or vice versa). This measurement denotes the number of alleles with the same frequency that would be required to achieve the level of heterozygosity at each locus for the given population, thereby correcting for the influence of rare alleles on allele number (Weir 1990). Averaged across all loci amplified, the effective number of alleles can be compared across populations, which is more informative than the mean number of alleles (Table 2). These results were compared with values calculated from published genotypes from eastern chimpanzees at Gishwati and Budongo Forest Reserves, from Gombe Stream National Park, and from western chimpanzees at Taï National Park. It should be noted that while the microsatellite loci amplified for Gishwati, Budongo, and Taï represent a subset of the 12 loci amplified in the Ugalla sample, 15 of the 16 microsatellite loci amplified for the Gombe sample were different (Table 1). Therefore, we conducted a second analysis to compare the mean expected heterozygosity of the same four microsatellite loci that were amplified in the samples from Ugalla, Gishwati, Sonso, and Taï. The statistical significance of differences in levels of expected heterozygosity was tested using the Mann–Whitney U test, which compares the averages of two distributions without assuming a normal distribution (Mann and Whitney 1947). The Garza-Williamson index (the number of alleles divided by the allelic range) was calculated for autosomal microsatellite loci data from each of the above populations; this index is expected to be low when a past bottleneck has occurred (Garza and Williamson 2001).

### *Y-chromosome*

For haploid data, a genetic diversity index analogous to expected heterozygosity ($H_e$) was calculated in the software *Arlequin version* 3.5 (Excoffier et al. 2005), based on the probability that two randomly chosen haplotypes within the Ugalla sample are different. These results were compared with published values from the eastern chimpanzees at Gishwati and Budongo Forest Reserves, and at Kibale and Semliki National Parks (Fig. 4). DNA extracts from the Gishwati and Ugalla males were amplified at 13 Y-chromosome loci, while the Ugandan chimpanzee males from Kibale, Budongo, and Semliki were compared using nine loci, although these nine represented a subset of the 13 loci amplified for the Gishwati and Ugalla samples. Of the 69 males identified in the Ugalla sample, eight males could not be assigned to a haplotype due to poor amplification and were not included. Individual males from Ugalla, identified by their Y-chromosome haplotype, were mapped using ArcGIS version 10.1 to investigate the geographic structure and distribution of the Ugalla haplotypes.

**Table 2** Summary statistics for all autosomal loci amplified in Ugalla population

| Autosomal locus | No. of individuals genotyped | No. of alleles | Observed heterozygosity $H_o$ | Expected heterozygosity $H_e$ | Effective no. of alleles $A_e$ $(1/(1-H_e))$ |
|---|---|---|---|---|---|
| D11s2002 | 110 | 5 | 0.73636 | 0.70535 | 3.39 |
| D2s1326 | 109 | 10 | 0.84404 | 0.82696 | 5.78 |
| D7s817 | 112 | 7 | 0.78571 | 0.81835 | 5.51 |
| D5s1470 | 110 | 6 | 0.66364 | 0.59153 | 2.45 |
| D6s1056 | 109 | 6 | 0.73394 | 0.70925 | 3.44 |
| D5s1457 | 112 | 6 | 0.625 | 0.64166 | 2.79 |
| D1s1622 | 98 | 6 | 0.5 | 0.50455 | 2.02 |
| D2s1329 | 110 | 6 | 0.76364 | 0.78348 | 4.62 |
| D3s2459 | 107 | 11 | 0.56075 | 0.55316 | 2.24 |
| D7s2204 | 108 | 8 | 0.76852 | 0.79143 | 4.79 |
| D14s306 | 109 | 9 | 0.74312 | 0.79284 | 4.83 |
| D16s2624 | 100 | 5 | 0.49 | 0.5097 | 2.04 |
| Mean | | 7.08 | 0.685 | 0.685 | 3.66 |
| SD | | 1.98 | 0.116 | 0.122 | |

### Genetic differentiation

We used *Arlequin version* 3.5 (Excoffier et al. 2005), to calculate $F_{st}$ values based on the Y-chromosome haplotypes for the five eastern chimpanzee sites, including Ugalla. The five sites were designated as five "populations" based on their location (i.e., Ugalla, Budongo, Kibale, Semliki, and Gishwati). It should be noted that the Budongo, Semliki and Gishwati haplotypes come from just one community each, and from two communities at Kibale. The Ugalla haplotypes were collected from across a large area and may include males from several communities. The $F_{st}$ values were based on the same nine microsatellite loci amplified from each of the five populations.

## Results

We analyzed DNA extracted from 237 samples collected across 624 km$^2$ of the Ugalla region. Of the 237 extracts, 197 were genotyped at the minimum number of eight loci necessary to ensure that identical genotypes were indeed from the same individual ($P_{IDsibs} \leq 0.001$). These 197 genotypes represented 113 unique individuals, comprised of 69 males and 44 females.

All of the 12 loci amplified for the Ugalla population were in Hardy–Weinberg equilibrium, and the mean expected heterozygosity (i.e., genetic diversity) of the Ugalla population was 0.68 (SD = 0.12) (Table 2), which is similar to other eastern chimpanzee populations, and lower than the mean observed heterozygosity for the western chimpanzee population at Taï (Mann–Whitney U, $P = 0.0169$) (Table 3). In fact, each of the eastern

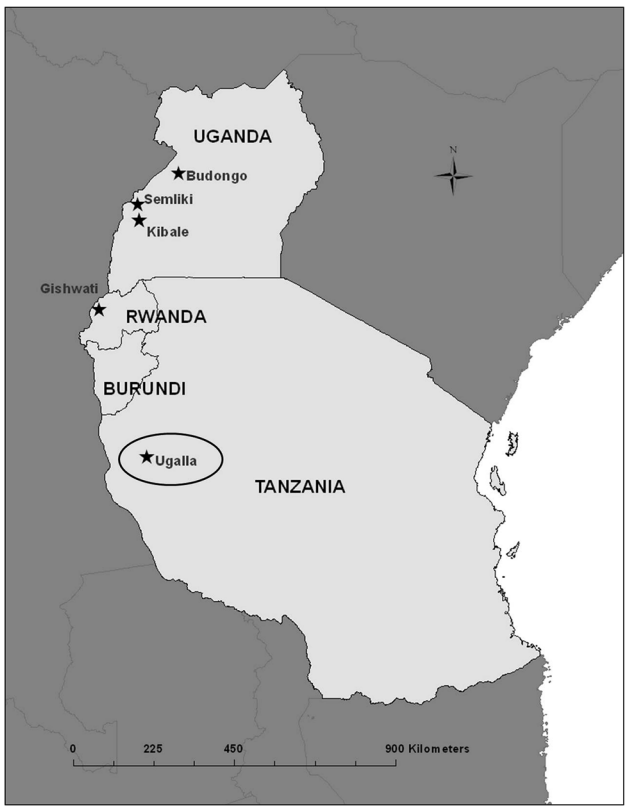


**Fig. 4** Map of East Africa showing the study sites used for comparison of Y-chromosome diversity in this study (represented by the *circle*)

populations have lower genetic diversity than the Taï population (Mann–Whitney U, Gombe: $P = 0.0033$, Gishwati: $P = 0.0028$, Sonso: $P = 0.0313$). The effective numbers of alleles for the Ugalla population was 3.17, again similar to

that found in other eastern populations, but lower than the Taï population (Table 3). When a subset of four microsatellite loci genotyped in four of the populations were compared, similar results were found, except that the heterozygosity of the Ugalla population was no longer significantly lower than the sampled population from Taï (Mann–Whitney U, $P = 0.1714$), although the Sonso and Gishwati population heterozygosities remained significantly lower than that of Taï, and not significantly different from the Ugalla mean heterozygosity (Table 3). The fact that this second analysis produced a similar pattern of lower autosomal diversity in the eastern populations, consistent with the results from the analysis including all microsatellite markers, suggests that the comparison of diversity measures inferred using somewhat different sets of microsatellite markers may be justified in this study. The Garza-Williamson index measure of diversity, which is sensitive to recent population decreases, was similarly low for all populations.

The Y-chromosome haplotype diversity of the Ugalla males was 0.27 (SD = 0.07), which is markedly lower than other eastern chimpanzee communities (Table 4). In addition to this low haplotype diversity, the most common haplotype, which was found in 52 of the 69 males in the Ugalla population, is distributed across the entire surveyed area of approximately 624 $km^2$, and was sampled at each of the six survey camps (Fig. 3).

As expected for a male philopatric species with limited male-mediated gene flow, the $F_{st}$ values for the Y-chromosome haplotypes of the five eastern chimpanzee populations are high (Table 5). As might be predicted due to geographic proximity (Fig. 4), the lowest levels of differentiation occurred between Kibale and the other two Ugandan populations, Semliki and Budongo. When compared to the other eastern chimpanzee populations, the samples from the Ugalla region result in the three highest $F_{st}$ values, approaching complete differentiation from three of the four populations with which they are compared. None of the Y-chromosome haplotypes were shared across any of the five eastern populations sampled.

## Discussion

Based on the autosomal genotypes of 113 individuals, the genetic diversity ($H_e$) and effective number of alleles ($A_e$) seen in the Ugalla population are low but similar to that of other surveyed eastern chimpanzee populations. Additionally, all of the eastern populations, including Ugalla, have significantly lower genetic diversity, as measured by average heterozygosity or effective number of alleles, than the individuals sampled from three western chimpanzee communities in the Taï National Forest.

These results contradict previous studies that investigated the demographic history and genetic structure of chimpanzees and demonstrated lower autosomal genetic diversity for western chimpanzees relative to eastern chimpanzees (Becquet et al. 2007; Fischer et al. 2006). It should be noted, however, that these previous studies were conducted on a sampling of captive individuals from varied geographic origins within the distribution of each subspecies. At least one study employing sequencing of autosomal DNA segments from individuals of captive origin found very similar nucleotide diversity ($\pi$) in eastern and western chimpanzee populations (0.08 vs. 0.09 %, respectively) (Yu et al. 2003). These results were further corroborated by findings of similar effective population sizes ($N_e$) for the two subspecies (Hey 2010), and evidence that both eastern and western chimpanzee populations experienced population bottlenecks prior to expansion to their current ranges (Wegmann and Excoffier 2010). The individuals used for comparisons within this study represent geographically localized samplings of limited numbers of communities, and may offer a more accurate

**Table 3** Comparison of autosomal microsatellite diversity across chimpanzee populations

| Population | No. of individuals genotyped | No. of loci[a] | Mean expected heterozygosity $H_e$ | Mean expected heterozygosity of coincident subset $H_e$ | Effective no. of alleles $A_e$ $(1/(1-H_e))$ | Mean Garza-Williamson Index ($M$) |
|---|---|---|---|---|---|---|
| Ugalla | 113 | 12 | 0.685 | 0.755 | 3.17 | 0.240 |
| Gishwati[b] | 19 | 12 | 0.701 | 0.693 | 3.34 | 0.244 |
| Sonso[c] | 49 | 9 | 0.716 | 0.761 | 3.52 | 0.253 |
| Gombe[d] | 39 | 16 | 0.702 | | 3.36 | Na |
| Ta[c] | 114 | 9 | 0.798 | 0.848 | 4.95 | 0.248 |

[a] Note that microsatellite loci amplified are not the same for each population (see Table 1)

[b] Calculated from genotypes published in (Chancellor et al. 2012)

[c] Calculated from genotypes published in (Lukas et al. 2005)

[d] From data published in (Constable et al. 2001)

**Table 4** Comparison of Y-chromosome gene diversity indices across several eastern chimpanzee communities, demonstrating the relatively low haplotype diversity value for the sampled Ugalla males (in bold)

| Community (population) | No. males typed | No. haplotypes | No. polymorphic loci | Haplotype diversity | SD |
|---|---|---|---|---|---|
| Ugalla[a] | 61 | 4 | 3 | **0.27** | 0.07 |
| Gishwati[b] | 12 | 3 | 2 | 0.62 | 0.09 |
| Sonso (Budongo)[c] | 16 | 4 | 4 | 0.74 | 0.06 |
| Kanyawara (Kibale)[c] | 10 | 3 | 4 | 0.62 | 0.14 |
| Ngogo (Kibale)[c] | 41 | 8 | 4 | 0.56 | 0.09 |
| Mugiri (Semliki)[c] | 6 | 3 | 4 | 0.60 | 0.22 |

[a] Males sampled from Ugalla likely come from several communities

[b] From data published in Chancellor et al. (2012)

[c] From data published in Langergraber et al. (2007)

**Table 5** Y-chromosome differentiation among eastern chimpanzee populations ($F_{st}$ values with 95 % bootstrap confidence intervals)

| | Ugalla | Gishwati | Kibale | Semliki | Budongo |
|---|---|---|---|---|---|
| Ugalla | 0 | | | | |
| Gishwati | 0.966 (0.91–1.00) | 0 | | | |
| Kibale | 0.841 (0.81–0.89) | 0.737 (0.68–0.81) | 0 | | |
| Semliki | 0.970 (0.94–0.99) | 0.899 (0.82–0.96) | 0.600 (0.50–0.67) | 0 | |
| Budongo | 0.941 (0.90–0.98) | 0.849 (0.75–0.94) | 0.670 (0.51–0.79) | 0.790 (0.65–0.92) | 0 |

representation of the relative genetic diversity present. The analysis of genetic samples from more centralized eastern chimpanzee populations would further improve our understanding of the levels of autosomal genetic diversity within the eastern subspecies; however, this would require samples from the DRC, and there are currently no chimpanzee study sites in this country or published genotypes available.

While autosomal genetic diversity measures are within the range of other eastern chimpanzee communities, the Y-chromosome diversity of the 61 typed Ugalla males is well below that of other eastern chimpanzee populations. In addition to a very low diversity index, the Y-chromosome haplotype present in the majority of the males (52 of 61 typed) was found in *all searches* across the entire 624 km$^2$ area surveyed, and the furthest distance between two males bearing this haplotype was approximately 38 km. This prevalence of one Y-chromosome haplotype across such a large area is unusual. While back mutation is a possible explanation for some haplotype sharing between chimpanzee communities, the reversion to a specific haplotype in so many individuals, as seen in Ugalla, is highly unlikely. Perhaps the unique demographic, geographic, and ecological conditions of Ugalla have led to this atypical distribution and diversity level of the Y-chromosome.

Ugalla is unusual among most of the chimpanzee sites mentioned here in that the region is classified as savanna-woodland, rather than forest. In socioecological models, ecological differences are predicted to effect changes in social structure (Wrangham 1979; Sterck et al. 1997; Isbell 1991), and the population density of the Ugalla region is estimated to be much lower than found in forested study sites (Moore and Vigilant in press; Moyer et al. 2006; Ogawa et al. 2007). This low population density may result in a low effective population size ($N_e$), which can reduce genetic diversity. Low population densities also could be responsible for a further restriction of male-mediated gene flow. For example, extra-group paternities may be less likely in such widespread communities. However, the Mugiri community at Semliki, is also a dry-habitat site, yet contains much higher Y-chromosome diversity than the Ugalla population.

In addition to a low effective population size ($N_e$), there may be other factors driving the paucity of Y-chromosome genetic diversity among the Ugalla males. Under the assumption that the population expansion into this region was relatively recent (Fischer et al. 2006), the Ugalla region may have been originally populated with a low number of males, or a group of highly related males. The latter is especially plausible, given the male philopatric social structure of chimpanzees. If the divergence of the eastern from the central subspecies was as recent as 77,000 years (Bjork et al. 2011), and Ugalla is the easternmost distribution, perhaps there has been little time for the accumulation of mutations to overcome the effects of genetic drift. A recent expansion into Ugalla would also explain the extraordinary prevalence of one Y-chromosome haplotype across the region.

A factor further exacerbating the restriction of male-mediated gene flow may be male reproductive skew, due to the hierarchical structure found among all habituated communities (Boesch and Boesch-Achermann 2000; Goodall 1986; Nishida 1968). In every paternity study of wild chimpanzees, researchers have found that male rank is highly correlated with paternity success (Boesch et al. 2006; Vigilant et al. 2001; Constable et al. 2001; Newton-Fisher et al. 2010), although recent research has demonstrated that the "socio-spatial relationship" between males and females also is an important predictor of reproductive success (Langergraber et al. 2013). The low population density at Ugalla (Moore and Vigilant in press; Moyer et al. 2006; Ogawa et al. 2007) may produce very different association and affiliation patterns among chimpanzee communities, but we currently know little about within-community grouping patterns of savanna-woodland chimpanzees. In a study at the savanna-woodland site of Mt. Assirik, Tutin et al. (1983) found that a higher proportion of the community remained together, and stayed together more frequently than at forested sites, and the authors suggested this may be driven by an increased risk of predation in the more open habitat. In contradiction to this hypothesis, Stewart and Pruetz (2013) found that nest groups at Ugalla were not larger than those of Fongoli, Senegal, a savanna-woodland site with no predators. Further behavioral studies of savanna-woodland chimpanzees are necessary to determine whether male reproductive skew is more or less pronounced in these dry, open habitats.

While male mating strategies may certainly contribute to low Y-chromosome diversity, the possibility that the Ugalla population is isolated also must be considered. The somewhat low autosomal diversity is consistent with isolation, as diploid genes are lost at a much slower rate than the haploid Y-chromosome. Ugalla may be bounded by the Malagarasi River to the north (but see Piel et al. 2013), and chimpanzee distribution ends to the east, perhaps leaving the Mpanda-Uvinza road to the southwest the only permeable boundary. While this is one of the only main roads in this remote part of western Tanzania, it remains a narrow dirt road that is rarely used (Moore, pers. obs.) and chimpanzees should be able to safely cross. In fact, the presence of chimpanzees was recorded very near the road in this study, and nests have been observed along the roadway (JJ Moore, pers. comm.). That said, these were the only chimpanzees found adjacent to the road during this 10 month survey, and human presence is concentrated along the roadway.

Further evidence of isolation is demonstrated by the exceptionally elevated *Fst* values of the sampled Ugalla males when compared to other eastern chimpanzee sites, and the absence of Y-chromosome haplotype sharing among any of the populations compared. Y-chromosome haplotypes from nearer sites such as Masito or Mahale, however, would provide a more informative comparison. In fact, evidence does exist that the Ugalla population has experienced gene flow with neighboring populations. A study of the maternally-transmitted mtDNA found that the Ugalla population was more closely linked to Mahale and two locations within the neighboring Masito region (located between Ugalla and Mahale) than they were to the chimpanzees of Gombe (Inoue et al. 2013), and this was suggested to demonstrate that the chimpanzees from Mahale to Ugalla could be considered one continuous population (i.e., "Greater Mahale"). Langergraber et al. (2011) also found that, based on mtDNA, the Gombe population was closer to Ugandan chimpanzee populations than to other Tanzanian populations, strongly suggesting that the Malagarasi River is a barrier to gene flow. In contrast to these findings, Rudicell et al. (2011), found the SIV strain identified in the Ugalla chimpanzees was most closely associated with the Gombe SIV strains, and found no SIV antibodies in Mahale chimpanzees. Although no chimpanzees from the Masito area were tested for the SIV antibody, that study does suggest that the Ugalla and Gombe chimpanzees may have exchanged genetic material at some time in the past, or that the SIV was introduced independently from the west side of Lake Tanganyika, via a northern and a southern route (Rudicell et al. 2011). Further evidence of gene flow between Gombe and Ugalla, across the Malagarasi River, has emerged based on chimpanzee fecal samples collected near the riverbank and the presence of a natural bridge comprised of rocks, where researchers were able to cross (Piel et al. 2013). The authors of this study also noted old feces on this 'bridge,' suggesting that chimpanzees are crossing the Malagarasi River.

Regardless of the historical distribution and gene flow patterns of the chimpanzees of western Tanzania, the Y-chromosomal genetic diversity estimated among the Ugalla males raises some concern for their continued persistence in this area, and studies of gene diversity among additional savanna-woodland populations would provide important context. Extremely low Y-chromosome haplotype diversity could be a first indication of isolation and degradation of the genetic diversity of this population. While there is a possibility that this low amount of diversity could be due to incomplete sampling, the number of males sampled and typed (61) is reasonable, and their distribution extensive. More research is needed to determine whether chimpanzees are crossing the Uvinza-Mpanda road, which would promote gene flow with other eastern chimpanzees of Tanzania. If this is occurring, this connection should be promoted and protected so that it remains open and freely available. The contribution to

Y-chromosome diversity by migration may be very low (i.e., 4.8 % as per Schubert et al. 2011), but the introduction of just one new haplotype would substantially increase the diversity of the Ugalla males. In addition, new areas of the Uvinza-Mpanda road should be identified, such as the area around the chimpanzees found nesting near the roadside that were identified in this study, that could be protected from increased human disturbance, and further ensure continued gene flow into the Ugalla population.

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