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Genetic evidence supports demic diffusion of Han culture

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The spread of culture and language in human populations is explained by two alternative models: the demic diffusion model, which involves mass movement of people; and the cultural diffusion model, which refers to cultural impact between populations and involves limited genetic exchange between them[1]. The mechanism of the peopling of Europe has long been debated, a key issue being whether the diffusion of agriculture and language from the Near East was concomitant with a large movement of farmers[1–3]. Here we show, by systematically analysing Y-chromosome and mitochondrial DNA variation in Han populations, that the pattern of the southward expansion of Han culture is consistent with the demic diffusion model, and that males played a larger role than females in this expansion. The Han people, who all share the same culture and language, exceed 1.16 billion (2000 census), and are by far the largest ethnic group in the world. The

expansion process of Han culture is thus of great interest to researchers in many fields.

According to the historical records, the Hans were descended from the ancient Huaxia tribes of northern China, and the Han culture (that is, the language and its associated cultures) expanded into southern China—the region originally inhabited by the southern natives, including those speaking Daic, Austro-Asiatic and Hmong-Mien languages—in the past two millennia[4,5]. Studies on classical genetic markers and microsatellites show that the Han people, like East Asians, are divided into two genetically differentiated groups, northern Han and southern Han[6,8], separated approximately by the Yangtze river[9]. Differences between these groups in terms of dialect and customs have also been noted[10]. Such observations seem to support a mechanism involving primarily cultural diffusion and assimilation (the cultural diffusion model) in Han expansion towards the south. However, the substantial sharing of Y-chromosome and mitochondrial lineages between the two groups[11,12] and the historical records describing the expansion of Han people[5] contradict the cultural diffusion model hypothesis of Han expansion. In this study, we aim to examine the alternative hypothesis; that is, that substantial population movements occurred during the expansion of Han culture (the demic diffusion model).

To test this hypothesis, we compared the genetic profiles of southern Hans with their two parental population groups: northern Hans and southern natives, which include the samples of Daic, Hmong-Mien and Austro-Asiatic speaking populations currently residing in China, and in some cases its neighbouring countries. Genetic variation in both the non-recombining region of the Y chromosome (NRY) and mitochondrial DNA (mtDNA)[13–16] were surveyed in 28 Han populations from most of the provinces in China (see Fig. 1 and Supplementary Table 1 for details).

On the paternal side, southern Hans and northern Hans share similar frequencies of Y-chromosome haplogroups (Supplementary Table 2), which are characterized by two haplogroups carrying the M122-C mutations (O3-M122 and O3e-M134) that are prevalent in almost all Han populations studied (mean and range: 53.8%, 37–71%; 54.2%, 35–74%, for northern and southern Hans, respectively). Haplogroups carrying M119-C (O1* and O1b) and/or M95-T (O2a* and O2a1) (following the nomenclature of the Y Chromosome Consortium) which are prevalent in southern natives, are more frequent in southern Hans (19%, 3–42%) than in northern Hans (5%, 1–10%). In addition, haplogroups O1b-M110, O2a1-M88 and O3d-M7, which are prevalent in southern natives[17], were only observed in some southern Hans (4% on average), but not in northern Hans. Therefore, the contribution of southern natives in southern Hans is limited, if we assume that the frequency distribution of Y lineages in southern natives represents that before the expansion of Han culture that started 2,000 yr ago[5]. The results of analysis of molecular variance (AMOVA) further indicate that northern Hans and southern Hans are not significantly different in their Y haplogroups ($F_{ST} = 0.006$, $P > 0.05$), demonstrating that southern Hans bear a high resemblance to northern Hans in their male lineages.

On the maternal side, however, the mtDNA haplogroup distribution showed substantial differentiation between northern Hans and southern Hans (Supplementary Table 3). The overall frequencies of the northern East Asian-dominating haplogroups (A, C, D, G, M8a, Y and Z) are much higher in northern Hans (55%, 49–64%) than are those in southern Hans (36%, 19–52%). In contrast, the frequency of the haplogroups that are dominant lineages (B, F, R9a, R9b and N9a) in southern natives[12,14,18] is much higher in southern (55%, 36–72%) than it is in northern Hans (33%, 18–42%). Northern and southern Hans are significantly different in their mtDNA lineages ($F_{ST} = 0.006$, $P < 10^{-5}$). Although the F_{ST} values between northern and southern Hans are similar for mtDNA and the Y chromosome, F_{ST} accounts for 56% of the total among-
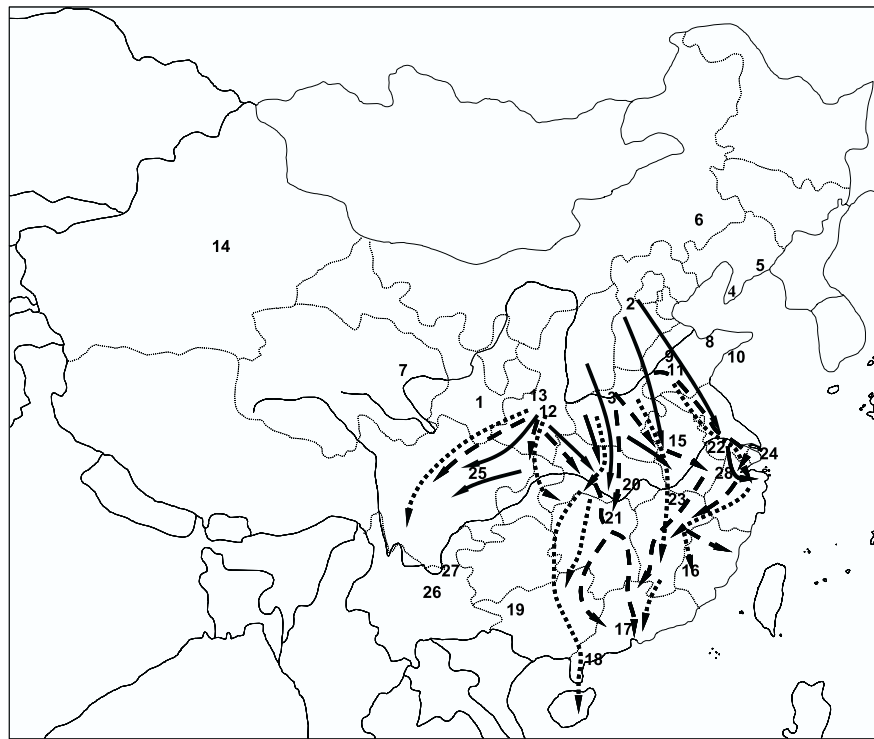
Figure 1 Geographic distribution of sampled populations. Shown are the three waves of north-to-south migrations according to historical record. The identifications of populations are given in Supplementary Table 1. Populations 1–14 are northern Hans, and 15–28 are southern Hans. The solid, dashed and dotted arrows refer to the first, second and third waves of migrations, respectively. The first wave involving 0.9 million (approximately one-sixth of the southern population at that time) occurred during the Western Jin Dynasty (AD 265–316); the second migration, more extensive than the first, took place during the Tang Dynasty (AD 618–907); and the third wave, including ~5 million immigrants, occurred during the Southern Song Dynasty (AD 1127–1279).

population variation for mtDNA but only accounts for 18% for the Y chromosome.

A principal component analysis is consistent with the observation based on the distribution of the haplogroups in Han populations. For the NRY, almost all Han populations cluster together in the upper right-hand part of Fig. 2a. Northern Hans and southern natives are separated by the second principal component (PC2) and southern Hans' PC2 values lie between northern Hans and southern natives but are much closer to northern Hans (northern Han, 0.58 ± 0.01; southern Han, 0.46 ± 0.03; southern native, -0.32 ± 0.05), implying that the southern Hans are paternally similar to northern Hans, with limited influence from southern natives. In contrast, for mtDNA, northern Hans and southern natives are distinctly separated by PC2 (Fig. 2b), and southern Hans are located between them but are closer to southern natives (northern Han, 0.56 ± 0.02; southern Han, 0.09 ± 0.06; southern native, -0.23 ± 0.04), indicating a much more substantial admixture in southern Hans' female gene pool than in its male counterpart.

The relative contribution of the two parental populations (northern Hans and southern natives) in southern Hans was estimated by two different statistics[19,20], which are less biased than other statistics for single-locus data[21] (Table 1). The estimations of the admixture coefficient (M, proportion of northern Han contribution) from the two methods are highly consistent (for the Y chromosome, $r = 0.922$, $P < 0.01$; for mtDNA, $r = 0.970$, $P < 0.01$). For the Y chromosome, all southern Hans showed a high proportion of northern Han contribution (M_{BE}: 0.82 ± 0.14, range from 0.54 to 1; M_{RH}: 0.82 ± 0.12, range from 0.61 to 0.97) (see refs 20 and 19 for definitions of M_{BE} and M_{RH}, respectively) indicating that males from the northern Hans are the primary contributor to the gene pool of the southern Hans. In contrast, northern Hans and southern natives contributed almost equally to the southern Hans' mtDNA

Table 1 **Northern Han admixture proportion in southern Hans**

Population	Y Chromosome		mtDNA	
	M_{BE} ($\pm$s.e.m)	M_{RH}	M_{BE} ($\pm$s.e.m)	M_{RH}
Anhui	0.868 ± 0.119	0.929	0.816 ± 0.214	0.755
Fujian	1	0.966	0.341 ± 0.206	0.248
Guangdong1	0.677 ± 0.121	0.669	0.149 ± 0.181	0.068
Guangdong2	ND	ND	0.298 ± 0.247	0.312
Guangxi	0.543 ± 0.174	0.608	0.451 ± 0.263	0.249
Hubei	0.981 ± 0.122	0.949	0.946 ± 0.261	0.907
Hunan	0.732 ± 0.219	0.657	0.565 ± 0.297	0.490
Jiangsu	0.789 ± 0.078	0.821	0.811 ± 0.177	0.786
Jiangxi	0.804 ± 0.113	0.829	0.374 ± 0.343	0.424
Shanghai	0.819 ± 0.087	0.902	0.845 ± 0.179	0.833
Sichuan	0.750 ± 0.118	0.713	0.509 ± 0.166	0.498
Yunnan1	1	0.915	0.376 ± 0.221	0.245
Yunnan2	0.935 ± 0.088	0.924	0.733 ± 0.192	0.645
Zhejiang	0.751 ± 0.084	0.763	0.631 ± 0.180	0.540
Average	0.819	0.819	0.560	0.500

M_{BE} and M_{RH} refer to the statistics described in refs 20 and 19, respectively. The standard error of M_{BE} was obtained by bootstrap with 1,000 replications. The proportions of contribution from northern Hans were estimated using northern Hans and southern natives as the parental populations of the southern Hans. It was assumed that the allele frequency in the southern natives remained unchanged before and after the admixture, which started about 2,000 yr ago, and the genetic exchange between northern Hans and southern natives has been limited. In fact, the gene flow from northern Hans to southern natives has been larger than that from southern natives to northern Hans; therefore, the level of admixture presented in this table is underestimated and is without proper adjustment. The demic expansion of Han would have been more pronounced than was observed in this study.

gene pool (M_{BE}: 0.56 ± 0.24 [0.15, 0.95]; M_{RH}: 0.50 ± 0.26 [0.07, 0.91]). The contribution of northern Hans to southern Hans is significantly higher in the paternal lineage than in the maternal lineage collectively (t-test, $P < 0.01$) or individually (11 out of 13 populations for M_{BE}, and 13 out of 13 populations for M_{RH}: $P < 0.01$, assuming a null binomial distribution with equal male and female contributions), indicating a strong sex-biased popu-

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lation admixture in southern Hans. The proportions of northern Han contribution (M) in southern Hans showed a clinal geographic pattern, which decreases from north to south. The Ms in southern Hans are positively correlated with latitude ($r^2 = 0.569$, $P < 0.01$) for mtDNA, but are not significant for the Y chromosome ($r^2 = 0.072$, $P > 0.05$), because the difference of Ms in the paternal lineage among southern Hans is too small to create a statistically significant trend.

We provide two lines of evidence supporting the demic diffusion hypothesis for the expansion of Han culture. First, almost all Han populations bear a high resemblance in Y-chromosome haplogroup distribution, and the result of principal component analysis indicated that almost all Han populations form a tight cluster in their Y chromosome. Second, the estimated contribution of northern Hans to southern Hans is substantial in both paternal and maternal lineages and a geographic cline exists for mtDNA. It is noteworthy that the expansion process was dominated by males, as is shown by a greater contribution to the Y-chromosome than the mtDNA from northern Hans to southern Hans. A sex-biased admixture pattern was also observed in Tibeto-Burman-speaking populations[22].

According to the historical records, there were continuous southward movements of Han people due to warfare and famine in the north, as illustrated by three waves of large-scale migrations (Fig. 1). Aside from these three waves, other smaller southward migrations also occurred during almost all periods in the past two millennia.

Our genetic observation is thus in line with the historical accounts. The massive movement of the northern immigrants led to a change in genetic makeup in southern China, and resulted in the demographic expansion of Han people as well as their culture. Except for these massive population movements, gene flow between northern Hans, southern Hans and southern natives also contributed to the admixture which shaped the genetic profile of the extant populations. □

Methods

Samples

Blood samples of 871 unrelated anonymous individuals from 17 Han populations were collected across China. Genomic DNA was extracted by the phenol-chloroform method. By integrating the additional data obtained from the literatures on the Y chromosome and on mtDNA variation, the final sample sizes for analysis expanded to 1,289 individuals (23 Han populations) for the Y chromosome and 1,119 individuals (23 Han populations) for mtDNA. These samples encompass most of the provinces in China (Fig. 1 and Supplementary Table 1).

Genetic markers

Thirteen bi-allelic Y-chromosome markers, YAP, M15, M130, M89, M9, M122, M134, M119, M110, M95, M88, M45 and M120 were typed by polymerase chain reaction-restriction-fragment length polymorphism methods[11]. These markers are highly informative in East Asians[23] and define 13 haplogroups following the Y Chromosome Consortium nomenclature[24].

The HVS-1 of mtDNA and eight coding region variations, 9-bp deletion, 10397 AluI, 5176 AluI, 4831 HhaI, 13259 $Hinc$II, 663 HaeIII, 12406 HpaI and 9820 $Hinf$I were sequenced and genotyped as in our previous report[22]. Both the HVS-1 motif and the coding region variations were used to infer haplogroups following the phylogeny of East Asian mtDNAs[18].

Data analysis

Population relationship was investigated by principal component analysis, which was conducted using mtDNA and Y-chromosome haplogroup frequencies and SPSS10.0 software (SPSS Inc.). The genetic difference between northern and southern Hans was tested by AMOVA[25], using ARLEQUIN software[26]. ADMIX 2.0 (ref. 27) and LEADMIX[21] software were used to estimate the level of admixture of the northern Hans and southern natives in the southern Han populations, using two different statistics[19–20]. The selection of parental populations is critical for appropriate estimation of admixture proportion[28,29] and we were careful to minimize bias by using large data sets across East Asia. In this analysis, the average haplogroup frequencies (for Y-chromosome or mtDNA markers, respectively) of northern Hans (arithmetic mean of 10 northern Hans) were taken for the northern parental population. The frequency of southern natives was estimated by the average of three groups including Austro-Asiatic (NRY, 6 populations; mtDNA, 5 populations), Daic (NRY, 22 populations; mtDNA, 11 populations) and Hmong-Mien (NRY, 18 populations; mtDNA, 14 populations). The geographic pattern of Han populations was revealed by the linear regression analysis of admixture proportion against the latitudes of samples[1,3].

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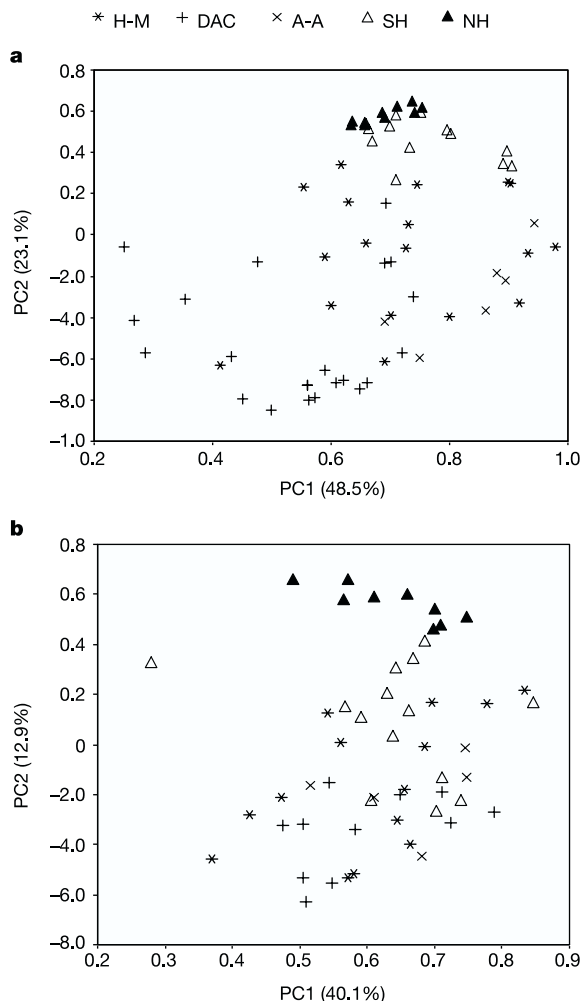
Figure 2 Principal component plot. **a**, **b**, Plots are of Y-chromosome (**a**) and mtDNA (**b**) haplogroup frequency. Population groups: H-M, Hmong-Mien; DAC, Daic; A-A, Austro-Asiatic; SH, southern Han; NH, northern Han.

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Post-mating clutch piracy in an amphibian

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Female multiple mating and alternative mating systems can decrease the opportunity for sexual selection[1–3]**. Sperm competition is often the outcome of females mating with multiple males and has been observed in many animals**[1,4–7]**, and alternative reproductive systems are widespread among species with external fertilization and parental care**[3,8–10]**. Multiple paternity without associated complex behaviour related to mating or parental care is also seen in simultaneously spawning amphibians**[11–15]** and fishes**[16]** **that release gametes into water. Here we report 'clutch piracy' in a montane population of the common frog *Rana temporaria*, a reproductive behaviour previously unknown in vertebrates with external fertilization. Males of this species clasp the females and the pair deposits one spherical clutch of eggs. No**

parental care is provided. 'Pirate' males search for freshly laid clutches, clasp them as they would do a female and fertilize the eggs that were left unfertilized by the 'parental' male. This behaviour does not seem to be size-dependent, and some males mate with a female and perform clutch piracy in the same season. Piracy affected 84% of the clutches and in some cases increased the proportion of eggs fertilized, providing direct fitness benefits both for the pirate males and the females[17]. Sexual selection— probably caused by a strong male-biased sex ratio—occurs in this population, as indicated by size-assortative mating; however, clutch piracy may reduce its impact. This provides a good model to explore how alternative mating strategies can affect the intensity of sexual selection.

Anuran amphibians have a wide diversity of reproductive modes, but external aquatic fertilization without parental care is the ancestral and most widespread strategy[18]. Only a few instances of multiple paternity have been demonstrated in frogs and those were considered to be the result of polyandrous matings, in which several males mate simultaneously with a female[11,13,14]. In the common frog *R. temporaria*, one of the most widespread Palaearctic amphibians[19], multiple paternity has been detected through allozyme analyses of tadpole kin groups, and was interpreted as being the consequence of high concentrations of spermatozoa in the water during simultaneous spawning[12].

R. temporaria is an explosive pond breeder that often reproduces immediately after the melting of the ice cover. Breeding is usually nocturnal[12,20] and males form large breeding aggregations. We monitored a high altitude population of common frogs in a medium-sized pond (540 m^2) during three consecutive breeding periods (2001–2003) in the central Pyrenean mountains, Spain (42°49′ N, 0°17′ W, about 2200 m above sea level). Breeding was exclusively diurnal due to low temperatures at night (Supplementary Information A), which permitted us to conduct detailed behavioural observations in the field and to measure and mark most individuals in this population. Males aggregated in a specific area of the pond, where clutches were also laid. Male density at the
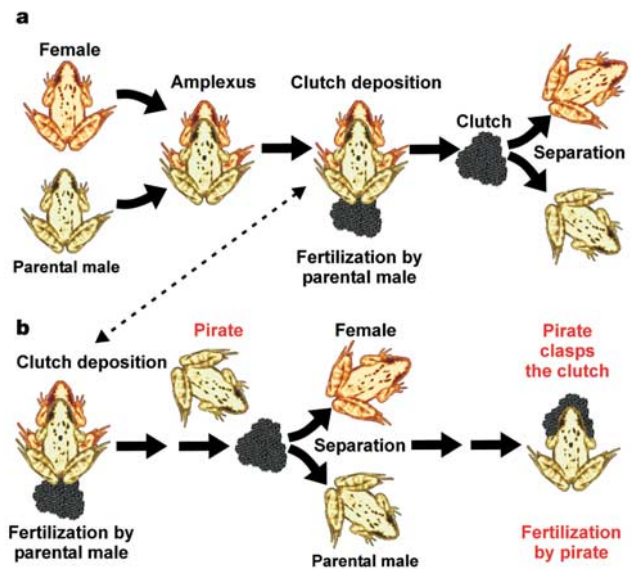

Figure 1 Schematic representation of mating systems in *R. temporaria*. **a**, Females arrive at the breeding ponds and are clasped in the axillary region ('amplexus') by a male (the 'parental' male). The female deposits a single, spherical clutch of eggs. The parental male simultaneously releases his sperm and thereby fertilizes the eggs externally. Subsequently both parents leave the clutch. **b**, 'Pirate' males search for freshly laid clutches, clasp them and release their sperm, sometimes crawling into the clutch to gain access to the internal eggs.