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FONDATO DA

PAOLO MANTEGAZZA

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Comitato scientifico di Revisione

Integrated Anthropology:

From Genetics to Ecology, Biodiversity, Conservation of Organisms, Cultures and Ethnicities

Selected Papers from symposia under the auspices of the Sokendai Advanced Sciences Synergy Program (SASSP) for International Collaboration

Held at:
Sokendai School of Advanced Sciences, Hayama, 2019
The University of Tokyo, Tokyo, 2020
National Museum of Ethnology (Minpaku), Osaka, 2020

Edited by
Hideyuki Tanabe, Francesca Bigoni, Giulia Dionisio and Roscoe Stanyon

Introduction to the Special Issue

The idea for a project on integrated anthropology originated with Hideyuki Tanabe. The possibility was first mentioned to Roscoe Stanyon and Francesca Bigoni at the symposium, *Genomics and Cell Biology of Primates*, which was held in Inuyama, at the Primate Research Institute of Kyoto University on March 23 and 24, 2018 in celebration of Hirohisa Hirai. Discussions continued in the following days at the University of Tokyo, between sessions of cherry blossom viewing, with the active contributions of Takafumi Ishida and Kazunari Matsudaira. The main thrust of the discussions was that more effort should be made to reconnect the various fields of anthropology. In its origins anthropology combined humanities and natural sciences. However, in more recent times this integration had become lost in Europe and Japan. In Japan especially due to the educational system which is divided into humanities and sciences, the departments of Anthropology were separated into cultural anthropology and physical anthropology. The project was envisioned to bring researchers together where they might meet, exchange ideas, and perhaps plant the seeds of future collaborations. It was thought that in this way anthropology could once again form a bridge between humanities and natural sciences.

Tanabe then took on the decision and the responsibility to explore funding opportunity with the School of Advanced Sciences, Sokendai. With perhaps more than good luck, the project was funded under the auspices of the International Collaboration Studies Program. The project was organized around three groups: 1. primatology, 2. genetics and molecular anthropology, and 3. ethnology, physical anthropology and museum studies. Tanabe's efforts lead to several symposiums. The first was the «*International Symposium, Integrated Anthropology: From Genetics to Ecology, Biodiversity, Conservation of Organisms, Cultures and Ethnicities*». Held on February 1, 2019 in Hayama. In 2020 two further symposia were held: one on January 27 at the University of Tokyo and another on January 31 at the National Museum of Ethnology (Minpaku) in Osaka.

One result of the symposiums of 2019 and 2020 was to establish a dialogue between the museums of ethnology in Florence and Osaka. Although these two museums have vastly different histories discussions between participants revealed that they actually shared many points of contact and mutual concerns, including engagement in collaborative projects with various indigenous people, decolonization,

both delicate and meaningful tasks.

The papers in this volume grew out of these symposia and represent a sampling of the topics presented and discussed. Altogether this volume brings together in contributions which illustrate the range of topics covered in the three symposia. We think it is particularly appropriate that these papers are published together in the Archivio per l'Antropologia e la Etnologia because the vision of anthropology of the journal founder (and the founder of anthropology in Italy) Paolo Mantegazza was a firm proponent of a holistic, view of anthropology were this young science «integrated» all fonts of knowledge and avenues of research to understand our species.

The dozen contributions begin with cellular biology and end with ethnological contributions and museology. First up is a detailed summary of nuclear architecture in relation to spatial radial distribution of chromosome territories and its characteristics in primates, mice, and chickens by the principle organizer Tanabe. This paper is followed by two papers on chromosome evolution in primates. One by Stanyon *et alii* which details the various rates and mechanism of chromosome evolution, concluding with a summary of possible implications for interpretations of recent human evolution. Capozzi *et alii* instead concentrate their attention on a review of chromosome heteromorphisms (polymorphism) and their significance for evolutionary processes. Matsudaira and Ishida present an original contribution on the complete mtDNA sequence of the Bangladesh rhesus macaque and develop hypothesis about the relationship of this population with Chinese and Indian rhesus macaques. Yonezawa *et al.* present an in depth paper on the origin of Japanese chicken breeds mainly from a mtDNA perspective, which fits into the growing discussion of the origin of domesticated species. Gojobori then illustrates how rare alleles can be informative about the origins of human populations and in particular how they can shed light on the population history of the Japanese population. Then Satta and Takahata bring new perspectives on a very classical topic of human biology and anthropology, lactase persistence. They analyze a mutation that is prevalent among Europeans and South Asians and conclude that the mutation arose in the Pontic Steppe. Tadakara *et al.* show how cultural anthropological insights can be helpful explaining the mode of Epstein-Barr virus transmission. At this point in the collection we arrive at a series of articles dealing more strictly with ethnology and museology. Nobayashi presents a detailed history of the origin of the National Museum of Ethnology (Minpaku) in Osaka which was originally formed around an exhibit at the Japan World

Exposition of 1970. Next, Bigoni and Roselli detail the anthropological interest and scientific voyages toward Japan which began in the mid 1800s which became the foundation of the Japanese collections of the Museum of Antropology in Florence. Bartolini Lucenti *et al.* then, using an Ainu Iku-bashui, show how 3D digitalizations can become an essential part of museology and diffusion of ethnological collections. The final paper by Ikeya illustrates how human culture is reflected and tranced in beads. This paper aimed to present a research framework for understanding the cultural history of relations between humans and beads. He shows how the investigation of beads sheds light on the cognitive revolution that has taken place in human history, including the formation and development of social networks through beads.

Through the three symposia there were many fruitful discussions and ideas for new collaborations that should be included in this volume, but due to time and space constraints, the volume is limited the current contents. We appreciate the support from the SOKENDAI Advanced Sciences Synergy Program (SASSP) for International Collaboration and believe this volume would shed light on and point the way forward for constructing a truly «Integrated Anthropology».

Roscoe Stanyon, Francesca Bigoni, and Hideyuki Tanabe

Chromosome territories and their spatial radial organization in the nuclear architecture

HIDEYUKI TANABE*

Parole chiave: territori cromosomici, 3D-FISH, organizzazione spaziale, distribuzione radiale, plasticità, architettura nucleare.

Riassunto — I cromosomi sono organizzati in «territori cromosomici» (chromosome territories o CTs) all'interno del nucleo cellulare. Dopo lo sviluppo di tecniche 3D-FISH, avvenuto negli anni '90, l'organizzazione spaziale dei CTs è stata indagata approfonditamente in molte specie. La ricerca ha indagato la questione di come i CTs siano localizzati nello spazio del nucleo cellulare, e di come vengano regolati il loro posizionamento e organizzazione. Studi degli ultimi due decenni hanno mostrato che la distribuzione radiale nel nucleo cellulare, dal centro ai margini del nucleo, ha diverse importanti caratteristiche: 1) È dipendente dalla misura fisica e dalla densità genica di ciascun CT, 2) Dal punto di vista evolutivo le regioni sinteniche CTs hanno la tendenza ad essere localizzate nella stessa posizione, 3) Lamina associated domains (LADs) influenzano la localizzazione dei CTs vicino alla periferia del nucleo, 4) Actin related protein 6 (Arp6), una componente ubiqua dei complessi che rimodellano i complessi di cromatina, influenza la globale distribuzione dei CTs, 5) Anche la variante Istone H2A.Z influenza la globale distribuzione radiale dei CTs non meno di Arp6. Collettivamente, la distribuzione spaziale radiale dei CTs mostra un'organizzazione non casuale. Tuttavia la mancanza di movimento rapido potrebbe avere anche significati funzionali. Se la localizzazione dei CTs fosse determinata da forze attive o passive, sarebbe possibile alterare la loro posizione, ma allo stesso tempo sarebbe difficile spostarli meccanicamente. In conclusione, a questo punto, le scoperte suggeriscono un ruolo funzionale della distribuzione radiale di CTs nell'architettura nucleare.

Key words: chromosome territories, 3D-FISH, spatial organization, radial distribution, plasticity, nuclear architecture.

Summary — Chromosomes are highly compartmentalized, into so-called «chromosome territories (CTs)» within the cell nucleus. After development of 3D-FISH techniques in the 1990s spatial organization of CTs was intensely investigated in many species. Many questions were investigated along with how CTs were spatially localized in the cell nucleus, including what regulated their arrangement and positioning. Studies over the last two decades showed that the radial distribution of CTs in the cell nucleus, from the center to the nuclear rim, has several important characteristics: 1) It is dependent on the physical size and gene density of each CT, 2) Evolutionarily syntenic regions of CTs have a tendency to be localized in the same position, 3) Lamina associated domains (LADs) influence CTs localization near the periphery of the nucleus, 4) Actin related protein 6 (Arp6), an ubiquitous components of chromatin

* Department of Evolutionary Studies of Biosystems, School of Advanced Sciences. The Graduate University for Advanced Studies, SOKENDAI, Hayama, Kanagawa, Japan.

remodeling complexes, influences the global radial distribution of CTs, 5) Histone variant H2A.Z also influences the global radial distribution of CTs but to a lesser extent than Arp6. Collectively, spatial radial distribution of CTs shows non-random arrangements with plasticity. However, it lacks rapid movement, and therefore, might have some weakly constrained functional significance. Whether localization of CTs is determined by active or passive forces, it is possible to alter the position of CTs but at the same time difficult to mechanically move CTs. Overall, the findings at this point suggest a functional role for radial distribution of CTs in the nuclear architecture.

Introduction: Historical views

Early concepts of the territorial organization of interphase chromosomes was first suggested by Carl Rabl in 1885 by observing salamander cell nuclei (Rabl, 1885). Later, in 1909 Theodor Boveri first coined the term «chromosome territories (CTs)» by observing nematoda live cell nuclei (Boveri, 1909). Since then, structural aspects of interphase chromosomes have been documented and several models for nuclear architecture were developed. Aided by great improvements in electron microscopy of the 1960s, David E. Comings proposed a non-territorial organization model (Comings, 1968). In the Comings model telomeres and centromeres were attached to the nuclear membrane but interstitial chromosomal regions were spread randomly, spaghetti like, within the nucleus. In contrast, the Cremer brothers Thomas and Christoph hypothesized that each chromosome had a territorial organization within the nucleus (for review, see Cremer and Cremer, 2010). They developed a laser UV-microirradiation system that induced very specific and localized genome damage. Measurements of the inflicted DNA damage were made through unscheduled DNA-synthesis in the presence of labelled tritium in the nucleus of Chinese hamster cells which were cultured until mitosis. Different types of damage were predicted according to how chromosomes were arranged in the nucleus. If chromosomes occupied distinct territories, localized damage would affect only a small subset of chromosomes, whereas if the chromatin fibers of each chromosome were randomly distributed throughout the nucleus, many chromosomes would be damaged. The black grains of radioactivity detected on a few Chinese hamster chromosomes showed that only a small subset of the chromosomes was damaged. This result conclusively supported the Cremer model and demonstrated the existence of CTs (Cremer et al., 1982). In the 1980s, the development of fluorescence *in situ* hybridization (FISH) techniques allowed the detection of specific DNA sequences and chromosome painting.

FISH and further developments in 3D-FISH imagining techniques enabled a direct visualization of CTs within the nucleus (Cremer and Cremer, 2001). A notable discovery was that distinctive different radial distributions in respect to location of CTs from the center to the periphery of the nucleus. When human chromosomes 18 and 19 were compared, particularly distinctive different radial distributions were found. These two chromosomes have almost the same physical size, but they have very different gene densities (Croft *et al.,* 1999, Cremer *et al.*, 2001). Analysis of human and chicken CTs using 3D-FISH technique also confirmed that the radial distribution of CTs depended on the sizes and gene densities of individual chromosomes (Cremer *et al.*, 2001, Habermann *et al.*, 2001). One question was if this characteristic was maintained during the cell cycle and/or changed during the process of development and differentiation. Other questions concerned the distribution difference in different cell types and/or species as well as other unknown factors that might influence the radial distribution. Over the last two decades answers to these questions and others have been provided by new evidence and findings. In 2019 the IMB (Institute of Molecular Biology, Mainz) Conference on «Chromosome Territories and Nuclear Architecture» was held to celebrate 75 years old birthday of Christoph Cremer, a pioneer of super-resolution microscopy, and it honored his long and outstanding career (http://www.imb.de/seminars-meetings/meetings/2019-imb-conference-chromosome-territories-nuclear-architecture). This conference was filled with excellent talks and presentations on the latest advancements in relation to CTs and nuclear architecture. Here my intention is to summarize with reference to latest findings, pertinent characteristics of radial nuclear arrangement of CTs focusing on their hierarchical structure.

Global Radial Distribution of Chromosome Territories: Evolutionary Views

Studies on chromosomes based on classical cytogenetics, namely, human clinical cytogenetics as well as evolutionary studies of the karyotype made remarkable progress after the development of FISH technique in the 1980s. Development of 3D-FISH technique was in part based on the hybridization with chromosome specific painting probes onto the cell nuclear preparations, in which the three-dimensional structure was conserved. This technique allowed researchers to visualize and map CTs in the single cell nucleus.

The spatial arrangement from the center of the nucleus to the

nuclear rim is called as radial arrangement/distribution. The radial arrangement of CTs depends not only on the gene density of individual chromosomes but also on the physical size. Larger chromosomes tend to be located in the «periphery» and smaller ones in the «interior» (Boyle *et al*., 2001). Figure 1 shows 3D-FISH analysis of human and other primate species. As mentioned before, human chromosomes 18 and 19 have almost the same physical size, but the gene density is extremely different (18: 3.3 genes/Mb; 19: 25.1 genes/Mb, according to http://www.ensembl.org/). These two chromosomes are spatially arranged discretely either near the nuclear membrane or inside the cell nucleus. Namely, chromosome 18 with low gene density is located in the «periphery», and chromosome 19 with high gene density is located in the «interior» (Fig. 1).

To examine whether this property is conserved during the primate evolution, 3D-FISH analysis was performed using the primate homologous painting probes corresponding to human 18 and 19 chromosomes hybridized onto the lymphoblastoid cells derived from the following species: chimpanzees, gorillas, orangutans, white-handed gibbons, Japanese macaques, cynomolgus monkeys, African green monkeys x Patas monkeys, cotton-top tamarins, common marmosets, and squirrel monkeys. The results showed that the radial arrangements of both human chromosome 18 and 19 homologues have been evolutionarily highly conserved in the examined primate species (Tanabe *et al*., 2002b, 2005) (Fig. 1). An interesting point is that the radial arrangements of human chromosome 19 homologues in chimpanzees and gorillas tend to be distributed slightly toward peripheral sides compared to those of humans and orangutans, which may be due to the effect of large subtelomeric repeats in chimpanzees and gorillas. In white-handed gibbons the homologous regions of human 18 and 19 chromosomes were divided into multiple subregions due to hyper karyotypic evolution, however, the radial arrangements of both human chromosome 18 and 19 homologous regions were also found to be evolutionarily highly conserved (Tanabe *et al*., 2002b).

There is another report for 3D-FISH analysis in early bovine embryos observed on the gene-rich chromosome 19 and gene-poor chromosome 20, which showed almost the same physical size and no significant differences of radial arrangements up to the 8-cell stage embryo. However, after zygotic genome activation, the radial arrangements of both CTs began to alter that gene-rich chromosome 19 localized in «interior» and gene-poor chromosome 20 localized in «periphery» (Koehler *et al*., 2009). Similarly, in mouse embryonic fibroblasts mouse

chromosomes 18 and 19 are almost same physical sizes but their radial arrangements are discretely different, gene-rich chromosome 19 (11.8 genes/Mb) localized in «interior» and gene-poor chromosome 18 (5.7 genes/Mb) localized in «periphery» (Malhas *et al.*, 2007). On the other hand, in case of comparative analysis on mouse chromosomes with corresponding to human chromosomes 18 and 19, it is hard to observe the radial arrangement of CTs using simple painting probes. Human chromosome 18 and 19 homologues of mouse chromosomes are divided into approximately 10 different subregions, which makes it difficult to compare them with painting probes. Then alternatively mouse BAC-DNA probes corresponding to human 18 and 19 chromosomes are highly promising to conduct comparative 3D-FISH analysis. Further studies will be clarified how degree of evolutionarily conserved spatial radial arrangement of chromosomal subregions.

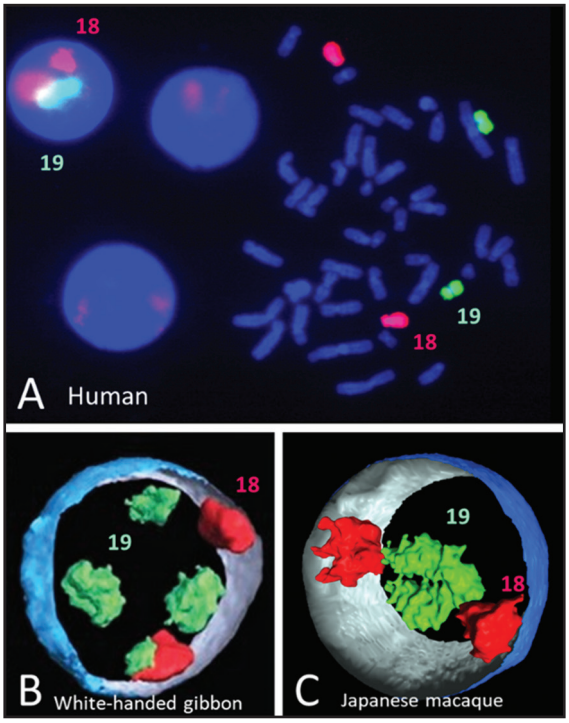

Fig. 1. *Visualization of human 18 (red) and 19 (green) chromosome territories.* A) *2D-FISH image of human metaphase spread and interphase nuclei derived from lymphoblast cells.* B-C) *3D reconstructed image of lymphoblast nucleus after 3D-FISH: B) White-handed gibbon,* C) *Japanese macaque.*

Radial Distribution of Chicken Macro- and Micro- Chromosome Territories

The chicken karyotype has *2n*=78, with a ZW sex determining system. The chicken chromosomes are morphologically divided into macrochromosomes (1-8, ZW) and microchromosomes (remained 30 pairs) (Fig. 2). Macrochromosomes have low gene density, are AT-rich, and replicate in the late S phase. In contrast, microchromosomes have high gene density (48% of all genes), are GC-rich (CpG islands are often found), and replicate in the early S phase. Analysis by 3D-FISH technique with 3 pooled probes of macro (1-5, Z), micro (19 pairs), and their intermediate sized (6-10) chromosomes revealed that the radial arrangements of macro, micro, and intermediate sized chromosomes were detected in clearly discrete regions as follows: macrochromosomes localized in «periphery», microchromosomes localized in «interior», and intermediate sized chromosomes localized in the intermediate radial zone (Habermann *et al*., 2001) (Fig. 2). This topology was observed in various kinds of chicken cells such as fibroblasts, lymphocytes, and neuron cells, suggesting a strong correlation between the physical size and gene density of chromosomes and their radial arrangements, as in human and primate cells. Human chromosome 18 and 19 homologues of the chicken chromosomes correspond to the macrochromosomes (chromosomes 2 and Z) and the microchromosome (chromosome 28), and their radial localization is in the «periphery» and the «interior», respectively. Therefore, it is considered that this topology has been evolutionarily conserved for more than 300 million years corresponding to the divergence time between human and chicken (Tanabe *et al*., 2002a).

Then, what kind of factors are involved in determining the radial arrangement? Here is an example that can address this question. Chicken DT40 cells are capable of performing target integration at a high rate, therefore, suitable for gene knockout analysis. We focused on one of the actin-related proteins (Arps) involved in the nuclear skeleton with highly conserved genes from yeast to human. Arp6 is a chromatin remodeling factor localized in the nucleus. As simple knockout of Arp6 gene would show severe lethality of the cells, conditional knockout experimental systems were constructed. Analysis by 3D-FISH technique using macro and micro chromosomes as probes, the global radial arrangement was examined. The results showed that the radial arrangements of macro and micro chromosomes were significantly impaired and disturbed in cell nuclei subjected to

conditional knockout by tet-on/off. This suggests that Arp6 is involved in the control of global radial localization of CTs (Fig. 2). In addition, similar conditional knockout experiments were performed on cells knocked-out of histone variant H2A.Z. As a result, global perturbation of radial arrangements was also observed in H2A.Z-KO cells, but the alteration effect was approximately half that of Arp6-KO cells.

The degree of perturbation of microchromosomes was more remarkable, often observed in close proximity to peripheral regions in both Arp6-KO and H2A.Z-KO cells (Maruyama *et al.*, 2012). These results might be due to the increased affinity of microchromosomes to the nuclear membrane. There are many factors that directly interact with Arp6 and H2A.Z, so the mechanisms of physically changing of the radial arrangements of macro and micro chromosomes have not yet been elucidated. The detailed molecular mechanisms of altering radial CTs will be revealed in the future.

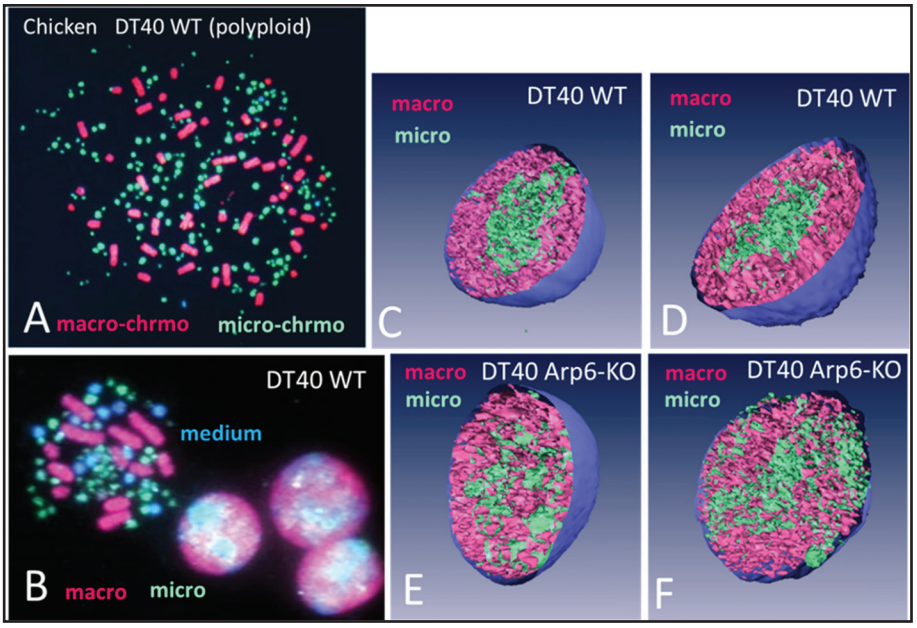

Fig. 2. *Visualization of chicken macro- (red) and micro-(green) chromosomes.* A) *2D-FISH image of chicken DT40 metaphase spread (polyploid cell) with macro- and micro-chromosomes.* B) *2D-FISH image of DT40 metaphase spread and interphase nuclei with macro-, micro-, and medium-chromosomes.* C-D) *3D reconstructed images of DT40 cell nuclei with macro- and micro-chromosomes.* E-F) *3D reconstructed images of DT40 Arp6-KO cell nuclei with macro- and micro-chromosomes.*

Plasticity in Radial Arrangement of Chromosome Territories

It has been reported that the radial arrangement of CTs is variable and has plasticity. The radial arrangement of CTs can change during the cell cycle, is dynamic at certain stages of development and differentiation. In proliferating fibroblasts human 18 CTs are localized to «periphery» like lymphocytes, but their radial localization alters to «interior» when the medium is serum starved into G0 phase. When serum is added to the medium again to return to the cycling state, the radial localization of 18 CTs returns to «periphery» (Bridger *et al*., 2000). Likewise, when Y chromosome is observed under the same conditions, the alteration dynamics of Y CT shows slightly opposite direction to that of 18 CTs (Bolzer *et al*., 2005). These results indicate that the radial arrangement of CTs has plasticity in response to physiological changes in intracellular metabolic status.

The differentiation processes can also alter the radial arrangement of CTs. In mouse T cells, mouse 6 CTs in undifferentiated cells show an intermediate radial arrangement, but are altered in «interior» in differentiated CD4 positive cells, and thereafter 6 CTs are altered in «periphery» in further differentiated CD8 positive cells (Kim *et al*., 2004).

Tissue-specificity of radial arrangement of CTs was also reported. Mouse 5 CTs are localized in «interior» in liver cells, localized in «periphery» in lung cells and localized in the intermediate radial zone in lymphocytes (Parada *et al*., 2004). In addition, as an example of the spatial arrangement of three genes, SNRPN, UBE3A, and GABRB3, which are localized in the genomic imprinting regions responsible for Prada-Willi and Angelman syndromes on the human chromosome 15, at 15q11.2-q12. SNRPN, UBE3A, and GABRB3 are linearly arranged in this order and the physical distance between SNRPN and UBE3A is 451kb and between UBE3A and GABRB3 is 1,298kb, respectively. These three genes were always spatially arranged as a three-dimensional triangle in both fibroblasts and lymphocytes (Kawamura *et al*., 2012). This spatial triangle structure seems to reflect the existence of TADs, which will be described later.

Alteration of radial arrangement of CTs during development and/or differentiation as well as during cell cycling could reflect differences in gene expression. However, a specific radial localization of CTs does not seem to be necessary for particular states of gene expression. Rather, the position of CTs are stochastically arranged from «periphery» to «interior». Heterogeneity in cells even in the same tissues was frequently

observed. It appears therefore, that in individual cells specific CTs may not be actively localized in specific radial zones but may be passively and stochastically arranged in such radial zones. This hypothesis is consistent with the report that radial arrangement is shaped by local gene density, not induced by differences in replication timing, changes in gene expression or epigenetic status (Küpper *et al*., 2007).

Plasticity in Relative Arrangement of Chromosome Territories: Kissing and Translocation

Plasticity in relative spatial arrangement of CTs has been found and specific association between particular two CTs is called as «chromosome kissing» (Cavalli, 2007). Chromosome kissing can be considered as a key event promoting chromosome translocations. One type of human liposarcoma in adipocytes is caused by the reciprocal translocation between chromosomes 12 and 16 indicated as t(12;16)(q13.3;p11.2). This translocation forms a chimeric gene between CHOP gene at 12q13.3 and TLS-FUS gene at 16p11.2 causing a liposarcoma tumor.

The relative spatial arrangement between 12 CTs and 16 CTs in preadipocytes maintains a distance without kissing, but when preadipocytes were induced to differentiate into adipocytes, the relative arrangement between 12 CTs and 16 CTs was altered and the pair 12-16 CTs kissing appeared with high frequency (approximately 95%). This kissing can trigger a specific t(12;16) translocation (Kuroda *et al*., 2004). Furthermore, it was found that the most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects the alteration of 12-16 CTs during adipogenesis. TCDD inhibits adipose differentiation at an early period in the process through the aryl hydrocarbon receptor (AhR). With TCDD exposure to preadipocytes, the relative arrangement of 12-16 CTs kissing was not detected. Thus AhR could be a key molecule involved in altering the relative arrangement of CTs (Oikawa *et al*., 2008).

Another case illustrated the relative distance of enhancer-to-promoter during development. Sonic hedgehog (Shh) encodes a signaling protein that plays pivotal roles in many processes during vertebrate development. Especially well studied is the effect on the limb-bud morphogenesis in early mouse embryo. Shh located on mouse chromosome 5 and its long-range enhancer MFCS1 located at approximately 1Mb upstream from Shh. Spatial distance between Shh and MFCS1 was examined in three regions of the limb-bud whether

Shh-MFCS1 kissing was observed at sites where Shh expression is active. The results showed that Shh-MFCS1 kissing was frequently observed at ZPA sites, however, it was unexpectedly observed at the anterior sites where Shh expression was not active. At the intermediate sites between ZPA and anterior sites where Shh expression was not active, Shh-MFCS1 kissing was not observed. Interestingly, Shh was localized on the surface of mouse 5 CTs at ZPA sites, whereas Shh was localized in the interior of mouse 5 CTs at both anterior and intermediate sites. These results indicated three different states of Shh on mouse 5 CTs, namely, active state at ZPA sites, silent state at intermediate sites, and poised state where Shh expression is not active but enhancer-promoter kissing occurs at anterior sites (Amano *et al.*, 2009). In summary dynamic plasticity in relative arrangement of CTs has been observed during development and/or differentiation.

Nuclear Architecture of Nocturnal and Diurnal Animals in Rod Photoreceptor Cells

Solovei *et al.* discovered a very interesting phenomenon concerning the nuclear architecture of rod photoreceptor cells. In general, heterochromatin in somatic cells is localized at the «periphery» and euchromatin at the «interior» of the nucleus, but in mouse rod photoreceptor cells, it was found that heterochromatin and euchromatin are reversely distributed (Solovei *et al.*, 2009). They investigated the distribution of both hetero- and euchromatin in rod cells derived from various kinds of mammals, and consequently found that normal distribution was observed in diurnal animals (conventional type), whereas inverted distribution was observed in nocturnal animals including mice (inverted type). The original type is considered as conventional type because in mouse embryo rod cells on day 0 of birth the conventional type was observed, then heterochromatin begins to separate from the nuclear membrane on 6th day of birth, and the inverted type was completed on 28th day suggesting the dynamic remodeling of nuclear architecture occured during the process of growth. In addition, LBR or LaminA/C contributes to bind the heterochromatin to the nuclear membrane in rod cells due to either LBR or LaminA/C expression is observed in diurnal animals but both genes are not expressed in nocturnal animals (Solovei *et al.*, 2013). Thus, it is possible to remodel the nuclear architecture from the conventional type to the inverted type in nocturnal animals.

In primates, the rod cells of owl monkeys have been well studied. It

was shown that owl monkeys have three types of megasatellite DNA sequences which are distributed near the center of nucleus and this nuclear architecture plays a fundamental role in light transmission in these noctural primates (Koga *et al.*, 2017). It seems logical that the radial arrangement of CTs in the inverted type rod cells should also be influenced. For example, CTs in rod cells corresponding to human 18 and 19 CTs should be localized invertedly. However, this arrangement or a reversed arrangement of CTs remains to be demonstrated. In future studies a series of analysis of the distribution of hetero- and euchromatin as well as radial arrangement of CTs would enable us to successfully link studies on the plasticity of nuclear architecture at cellular levels to studies on adaptation of low light environment at the ecological levels. To understand the mechanisms for reorganization of radial arrangement of hetero- and euchromatin, mathematical approaches have been applied and physical factors have been elegantly solved by the phase-field method (Lee *et al.*, 2017).

Lamina Associated Domains Influence Peripheral Localization of Chromosome Territories

The nuclear periphery is constituted by a distinct set of inner nuclear membrane (INM) proteins such as lamin B receptor (LBR) and emerin, as well as nuclear lamina, which is underlying inside of INM. Nuclear lamina interacts with transcriptional repressors, providing an environment to suppress gene expression (Reddy *et al.*, 2008). LBR and lamin B1/B2 or lamin A/C are essential for the binding of heterochromatin to tether CTs to the nuclear periphery. Nuclear lamina interacts with genomic regions, called lamina associated domains (LADs). These were identified by DamID techniques and a high-resolution map for the entire human genome was generated (Guelen *et al.*, 2008). From DamID-map of lamin B1 human chromosome 18 has a much higher density than that of chromosome 19, which is consistent with the peripheral localization of 18 CTs. Other peripherally localized chromosomes such as 8, 13, 21, and X show slightly higher density of LADs. Further, radial arrangement of mouse 18 CTs, localized at «periphery» in mouse fibroblasts, were altered to be shifted away to the «interior» side in lamin B1 knockout mouse cells (Malhas *et al.*, 2007). In addition, nuclear envelopathies such as progeria and laminopathy disease lead to aberrations on the nuclear morphologies. Periphery localized CTs are moved to interior side. An example is the case of fibroblasts derived from a cardiomyopathy patient possessing a point

mutation (E161K) in LMNA gene. This gene, encodes lamins A and C, and showed mislocalization of radial arrangements of 13 CTs from periphery to interior (Mewborn *et al*., 2010). Taken together, LADs contribute to modulate CTs for radial localization at the peripheral regions near INM through associating with nuclear lamina.

Hierarchy in Nuclear Architecture: Future Perspective

As mentioned above, CTs are compartmentalized and highly organized within the nucleus. Each human cell nucleus harbors 46 chromosomes that are highly packaged and efficiently functioned in appropriate time and space. So far, higher order structural models delineating this feature of nuclear architecture have been presented. In the pioneering days, interchromosome domain (ICD) model in 1993 and chromosome territory-interchromatin compartment (CT-IC) model in 2001 were proposed (Cremer *et al*., 1993, Cremer and Cremer, 2001). The important point underlying these models was the focus on the interchromatin compartment (IC) between CTs. This provided an IC for passaging and accumulating the various nuclear machinery factors in relation to transcription, splicing, replication, and repair. Active genes lie at the surface of chromatin domains whereas inactive genes are placed at deep inside of chromatin domains. Later, CT-IC model was updated in 2015 as active nuclear compartment (ANC) and inactive nuclear compartment (INC) network model (Cremer *et al*., 2015). The ANC is a composite structural and functional entity of a 3D-channel network in perichromatin region (PR) with transcriptionally competent decondensed chromatin connecting to the nuclear pores and the INC is represented by the compacted core of chromatin domain clusters (CDCs) enriched in markers for silent chromatin. The evidence for existing CDCs and PR, which harbors hnRNP transcripts at the surface of CDCs, was demonstrated by using transmission electron microscopy as well as 3D-SIM-based DAPI intensity classification (Albiez *et al*., 2006, Smeets *et al*., 2014).

On the other hand, in 2006 cryo-FISH technique was developed for the observation of chromatin nanostructure. This method showed that the boundary regions of CTs were occupied by an intermingling structure, not by IC, suggesting CTs significantly intermingle with each other, and proposed as the interchromosome network model (Branco and Pombo, 2006). This model hypotheses that chromosome intermingling is influenced by transcription-dependent interactions between CTs that correlates with translocation potential, and that transcription factories

can control simultaneously multiple chromosomal regions with not only cis-regulation but also trans-regulation beyond chromosomes (Fraser and Bickmore, 2007). The interchromosome network model does not consider the need of IC, which contradicts the observation of electron microscopy and, at least in part, the CT-IC and ANC-INC network models (Albiez *et al*., 2006, Cremer and Cremer, 2010).

Meanwhile, several technologies have made dramatic advances including next-generation sequencing, super-resolution microscopy, and chromosome conformation capture (3C) (Dekker *et al*., 2002). The 3C technique can detect specific genomic interactions between chromatins, namely between one region and another (one to one). Other techniques extent 3C, such as 4C (one to all), 5C (many to many), and Hi-C (all to all) (Lieberman-Aiden *et al*., 2009, Kempfer and Pombo, 2020). Hi-C map can provide the genome-wide chromatin contacts at a length scale of hundreds kb to a few Mb. This method becomes a basis for introducing further new concepts of topologically associating domains (TADs) and compartments (Dixon *et al*., 2012). TADs are basic structural units that include expressed genes and their enhancers with approximately 1 Mb (varying from 40kb to 3Mb) delimited by insulators, CCCTC-binding factor (CTCF) sites, at both ends. TADs consist of bundled clusters which form smaller chromatin loop domains as a substructure detected by microscopy. Several TADs form a mutually exclusive region of A and B compartments. Compartment A consists of euchromatin and shows gene dense with highly transcribed, early replicated active genes and harbors activating chromatin marks (i.e. H3K4me3 and H3K27ac). Compartment B consists of heterochromatin, has less transcribed, late replicated genes and harbors repressive chromatin marks (i.e. H3K27me3 and H4K20me).

In brief, the cell nucleus is occupied by CTs, CTs are partitioned into A and B compartments, and A-/B-compartments are partitioned into TADs, which are further partitioned into chromatin loops, chromatin, and DNA fibers. The hierarchical order is established as the essence of nuclear architecture as follows: cell nucleus - CTs - A-/B-compartments - TADs - chromatin loops - chromatin - DNA fibers

Radial arrangement of CTs depends on the complex accumulation of all the hierarchical components reflecting various tissue-specific dynamics through development and differentiation. It also relects rapid physiological responses and long-termed evolutionarily adaptations. It has been reported that TADs have highly evolutionarily conserved characteristics (Lazar *et al*., 2018). Among the hierarchical components TADs might be important units affecting positioning of

CTs. Accordingly, newly ANC-INC network model has been presented for depicting global nuclear architecture based on involving recent concepts of TADs and compartments (Cremer *et al*., 2015).

Collectively, spatial radial organization of CTs shows non-random arrangements with high plasticity. However, they are without rapid movement, suggesting that localization of CTs at a certain radial zone will be weakly constrained. The spatial radial organization of CTs reflects perhaps passively the accumulated dynamics of all the hierarchical components, especially in the interior localization. Peripheral localization of CTs appear more stronger constrained in association with LADs and/or Arp6 regulation. These factors might provide important hints to elucidate biological functions and to understand mechanisms for localizing or moving CTs to a certain radial zone in the nuclear architecture. In future, integrated analyses of 4D nucleome (Cremer *et al*., 2015) combined with super-resolution microscopy, CRISPR-imaging, AI technologies, theoretical approaches with mathematics, and anthropological knowledge in relation to evolutionary phylogenetic relationships might shed light on providing the basis for significant progress in understanding the organization of nuclear architecture.

Corresponding author: tanabe_hideyuki@soken.ac.jp

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Tempo and Mode in Primate Evolution

ROSCOE STANYON* ORONZO CAPOZZI*
NICOLETTA ARCHIDIACONO** FRANCESCA BIGONI***
MARIANO ROCCHI**

Parole chiave: citogenetica, riarrangiamenti cromosomici, speciazione, definizione di specie, primate, evoluzione umana, ibridazione.

Riassunto — Tassi e meccanismi di evoluzione dei primati sono discussi entro una cornice storica prestando particolare attenzione alla citogenetica e alla genomica dei primati. Nella letteratura classica, le fissioni e le fusioni centromeriche erano considerate i più importanti meccanismi nella promozione dell'evoluzione dei cariotipi dei primati, ma erano stati individuati anche altri meccanismi come inversioni, delezioni e duplicazioni e, più recentemente, gli spostamenti di centromeri. Poiché i riarrangiamenti cromosomici coinvolgono un alto carico genetico, si pensava che la speciazione avvenisse soprattutto in popolazioni limitate in numero, tempo e spazio. Riconoscere che l'evoluzione del genoma procede a tassi ampiamente variabili è stato un importante fattore nel passaggio da un'analisi fenetica ad un'analisi cladistica delle relazioni filogenomiche. La sostituzione del concetto biologico di specie con un concetto filogenetico ha portato ad un incremento di tre volte nel numero di specie di primati e ad un significativo aumento nel numero di specie attribuite al genere *Homo*. Se accettiamo che l'isolamento riproduttivo non è più una caratteristica che definisce la specie, relazioni e processi evolutivi non possono essere più rappresentati dall'icona dell'albero, ma vengono meglio descritti come una rete o un flusso di correnti che si intrecciano riflettendo una situazione di diffuso interbreeding.

Key words: cytogenetics, chromosome rearrangements, speciation, species definition, primate, human evolution, hybridization.

Summary — Evolutionary rates and mechanisms of primate evolution are discussed within an historical framework paying particular attention to primate cytogenetics and genomics. Classically, centromeric fissions and fusions were considered the most important mechanism promoting the evolution of primate karytoypes. Other mechanisms such as inversions, deletions and duplications and more recently centromere shifts were recognized. Since chromosome rearrangements result in a high genetic loads, speciation was thought to mostly occur in populations restricted in number, time and space. The recognition that genome evolution proceeds at widely varying rates was an important factor in the shift from phenetic to cladistic analysis of phylogenomic relationships. The replacement of the biological species concept with the phylogenetic species concept has led to a threefold increase in the number of primate species and a significant increase in the number of species attributed to the genus *Homo*. If we accept that reproductive isolation is no longer a defining characteristic of species, evolutionary relationships and processes can no longer be

* Dept. of Biology, University of Florence, Italy.
** Dept. of Biology, University of Bari Aldo Moro, Italy.
*** Museum of Anthropology and Ethnology, Natural History Museum, Florence, Italy.

represented as a tree. Instead, relationships might better be represented as a network or braided stream of pervasive interbreeding.

Tempo and mode are classical questions about evolution most famously and explicitly explored by the paleontologist George Gaylord Simpson (1944) in the light of issues previously raised by Dobzhansky (1937). Tempo deals with evolutionary rates and mode deals with process and mechanisms, the way in which evolution works. Ultimately, the question is about the origin of species. Here I consider both historical and current ideas about tempo and mode in primate genome evolution, paying particular attention to cytogenetic perspectives with the hopes of shedding some light on current problems of primate taxonomy and phylogeny including humans.

Mode in Chromosome Evolution

We can begin with the 1916 paper by William Robertson in which he discussed *whole-arm translocations* or *centric-fusion translocations*, now known as Robertsonian translocations. This mechanism also includes centric fissions and was responsible for changes in diploid numbers. To counting diploid numbers (2n) Matthey (1945) added a count of chromosome arms and coined the term *nombre fundamental* (fundamental number FN). By the time Matthey published his paper it had become very well accepted that FN and chromosome morphology could also change by pericentric inversions. The centromere was central to the mode of chromosome evolution but to his credit Matthey recognized that interpretations of chromosome modes were complete conjectures until the nature of the centromere was better understood.

Various modes were recognized that could change chromosome morphology and promote chromosome evolution. Classically, various modes mechanisms (modes) of chromosome rearrangements were recognized. These structural rearrangements (mutations) can be classified into two types: 1. Interchromosomal such as translocations, and 2. Intrachromosomal which includes inversions, both paracentric and pericentric. We can add deletions, duplications and the recently discovered neocentromere formation (see Capozzi *et al.* in this volume). A common theme linked all types of structural rearrangements both intra- and interchromosomal is that in meiosis they resulted in some 50% unbalanced gametes. It was concluded that all chromosome rearrangements obligatorily caused an incredibly high genetic load. The

theoretical implications were preponderant. Speciating populations must be small in number, restricted geographically and temporally brief. These are the integral premises of both the demic and the punctuated equilibrium models of speciation. Some model of human chromosome evolution took the demic model to extremes. Chiarelli (1967) proposed that the reduction in human diploid number from 48 to 46 (due to the fusion origin of what we now know was human chromosome 2) must have occurred in just a very few generations accomplished by severe inbreeding between father and daughters. The demic model also fit well with the synthetic theory of evolution most often associated with Ernst Mayr (1942). Here geographic isolation interrupts gene flow. When the speciating population is relatively small, genetic drift, consanguinity and selection were thought to reformulate the gene pool in a brief lapse of time. However, the demic and punctuated equilibrium models seemed to contradict some of the syntenic theory tenets and especially some of Simpson's conclusions. For example, that mutations with small effects are much more common that those with large effects, selection was not a mostly negative process and was more effective in large rather than small populations (Fitch and Ayala, 1994). In respect to tempo, Simpson showed that rates varied considerably.

For many cytogeneticists the demic model had clear implication at the taxonomic level, summed up in the expression «one karyotype one species» because selection was viewed as overwhelmingly negative. In the rare cases in which within species variation was found it was considered evidence of hybridization, most often in captivity - the almost exclusive source of the small number of samples analyzed. When Seuanez (1979) showed that Bornean and Sumatran orangutans differed by a pericentric inversion in chromosome 2 (homolog to human 3) it was taken as the first genetic evidence that Bornean and Sumatran orangutans belonged to different species. Sea level changes and alternating Sunda shelf exposure and submersion also appeared to nicely fit the geographic model of speciation. The inversion was judged sufficient to create lowered fitness in hybrids. Captive breeding programs began avoiding cross breeding Bornean and Sumatran orangutan and eliminated hybrid individuals from reproduction (Seuanez, 1982).

The whole genome sequencing of the orangutan (Locke *et al*., 2011) supported the existence of two species but contradicted the demic/geographic model of speciation. The effective population number, N_e, of speciating orangutan populations was estimated as sufficiently large (somewhere between 7000-11000 individuals) to avoid most effects of

genetic drift.

Tempo in Chromosome Evolution: *Phenetic vs Cladistic analysis in Cytotaxonomy*

Classic cytogenetic analysis based on gross staining and banding was essentially phenetic. The more different the karyotypes of two species were the more distant they were. Phenetic analysis gained support from the molecular clock analysis of evolutionary distance, which postulated that since differences were stochastic, times of divergence were correlated with the amount of genome evolution. It was a convenient simplification that provided a false sense of confidence in using chromosome for constructing taxonomy and phylogeny. Although this simplification often fits observation and appears logical, acute observers had noted that 2n number could differ radically even between closely related species for example the difference between recently diverged Muntjac deers (*Muntiacus reevesi* 2n=46 vs *Muntiacus muntjac* 2n=6♀/7♂; Yang *et al*., 1995). Chromosome banding reconstruction of human and African apes showed that after divergence from a common ancestor about 6 million years ago more rearrangements were incorporated in the African apes than in the human lineage. These data raised the seeming outlandish conclusion, since confirmed at the sequence level, that the African apes had more highly evolved genomes than humans (see Fig. 1).

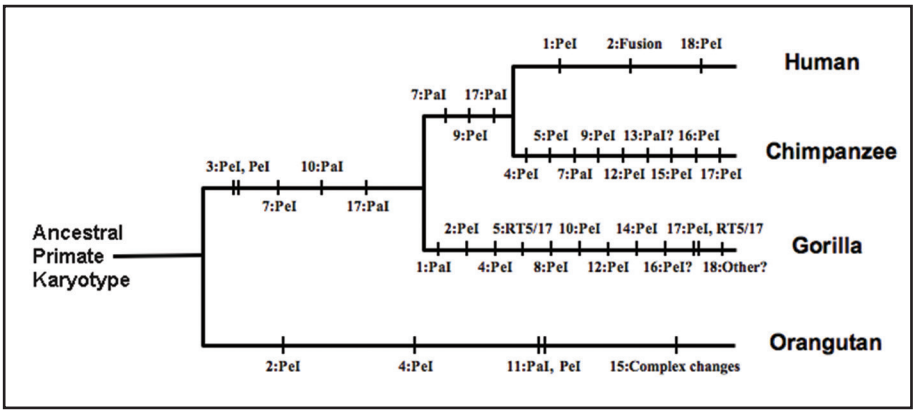

Fig. 1. *Chromosome rearrangements in the evolution of humans and great apes seen at the cytogenetic level using chromosome banding. It was clear even from banding analysis that chromosome evolution proceeded at various rates and that the African apes had more evolved karyotypes than humans. PeI=pericentric inversion, PaI=paracentric inversion, RT=reciprocal translocation, and the number refers to the human homolog.*

Interpretative problem arose because before the introduction of molecular methods into cytogenetics comparison were based solely on morphology and it was easy to confuse analogy with homology; that is confusing similarity due to convergence with that due to genealogy or descent. This led some primate cytogeneticists to untenable conclusions concerning the phylogenetic position of lesser apes. Chiarelli (1963) on the basis of similar morphology considered gibbons more closely related to colobine monkeys while others on the basis of a banding even stated that macaques were phylogenetically closer to humans than gibbons (Bernstein, 1980).

Cytogenetic comparisons were put on firmer ground when molecular methods made comparisons at the DNA level possible. Among the various molecular cytogenetic methods chromosome painting has enjoyed the most widespread application and utility. Instead of phenetic comparison, molecular methods made cladistic analysis of chromosome rearrangements possible. Polarity of evolutionary change could be established through the principle of parsimony, commonality, and outgroup comparison. For example, when a chromosome synteny was found intact in an array of different species this condition was considered *ancestral* for all those species. Derived rearrangements and *associations*, which are formed when a single target chromosome is hybridized by different whole chromosome probes of the index species, could be treated as traits or cladistic markers.

The first papers mapping chromosome homology between species using molecular cytogenetics methods (chromosome painting) were on Hominoids (chimpanzee, gorilla, orangutan, gibbons (Jauch *et al*., 1992) and Japanese macaques (Jauch *et al*., 1992; Wienberg *et al*., 1992). The painting revealed that humans, the great apes and Old World monkeys were highly conserved whereas the gibbons were radically rearranged. The conclusions were profound: the molecular clock did not keep time for chromosome evolution. The vastly different evolutionary rates made phenetic analysis (similarity) virtually useless, it does not provide evolutionary relationships (Marks, 1982). Even within different primate groups such New World or Old World monkey highly different rates of evolution were found. Some lines had accelerated chromosome evolution while others had slow evolution and were far better conserved. For example, in the platyrrhines, the genus *Aotus* (Owl monkeys) like gibbons is characterized by highly accelerated chromosome evolution while *Chiroptes* and *Cebus* are well conserved (Stanyon *et al*., 2004, 2011). If we survey the entire primate order we can find instances of highly accelerated evolution in lemurs

and lorids, in various New World monkey genera, in gibbons and to a lesser extent Cercopithecini (see Fig. 2) (Stanyon *et al.*, 2005; Tolomeo *et al.*, 2020).

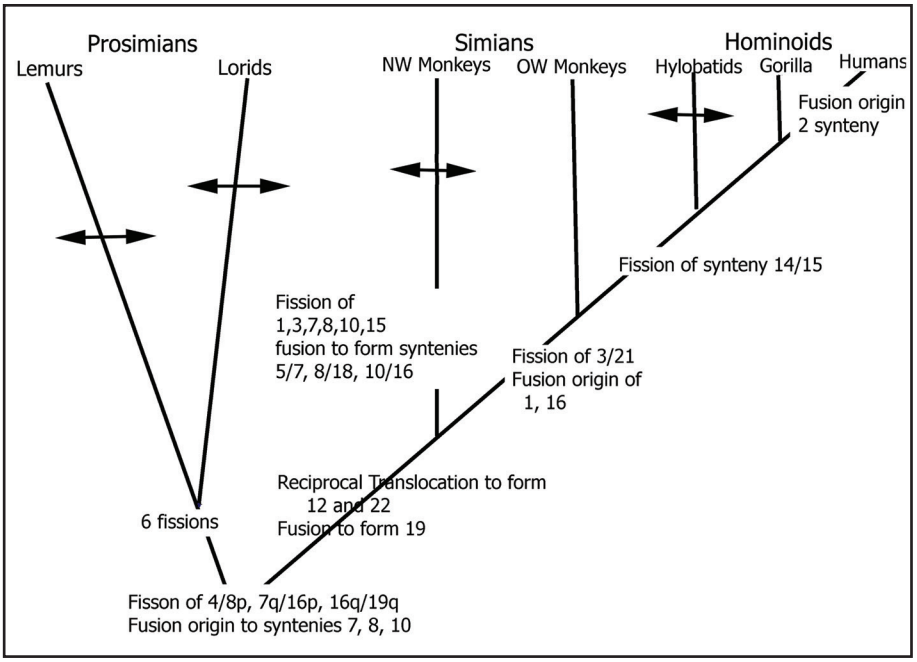

Fig. 2. *A simplified primate phylogeny. The horizontal arrows indicate rapid chromosome evolution. Although primates have generally well conserved genomes there have been bursts of rapid chromosome evolution: in the line leading to prosimians, New World monkeys and gibbons.*

Why do some evolutionary lines experience different rates (tempo) of chromosome evolution?

Apparently duplicons promote chromosome rearrangements. Many duplicons exhibit >95% sequence identity span large genomic distances (1-100 kb). An increasing number of human diseases are known to involve unstable genomic regions composed of duplicons and the acquisition of genomic instability is a crucial step in the progression of human cancers. Researchers have reported that these same areas are targets for rapid evolutionary turnover in primates. The conclusion is that duplicons, mobile elements and transposons apparently are involved in rearrangements in both evolution and disease.

The gibbon genome provides an intriguing example of the connubial relationship of duplicons and rapid chromosome evolution. The sixteen species of lesser apes (Hylobatidae) are divided into four genera

each with a distinctive karyotype at every level of analysis (*Holoock* 2n=38, *Hylobates* 2n=44, *Symphalangus* 2n=50 and *Nomascus* 2n=52). The Hylobatidae are the foremost example of rapid chromosome evolution in all of the primates, on a similar level to that found in murid rodents (Carbone *et al*., 2012).

The genome of Hylobatidae contains all previously transposable elements reported in other catarrhines (Old World monkeys and apes). One exception is LAVA a unique transposable sequence found only in gibbons (Carbone *et al*., 2012). Twenty-two lava subfamilies were described and 52% of loci were shared among the four genera. These transposons are significantly associated with rearrangement breakpoints and they are responsible for the rapid chromosome evolution found in these primates. At the opposite end of the continuum is the highly conserved orangutan genome. Among the hominoids this is the best example of slow genome evolution. Here again the presence of absence of duplicons explains why the orangutan genome evolves so slowly. The major transposable sequences in great apes are SVA, L1 and ALU. The orangutan genome has a much-reduced number of ALU sequences which has limited the effect of a wide variety of repeat-driven mutational mechanisms. The major number of ALU repeat for example in the chimpanzee lineage has determined a much higher evolutionary rate (Carbone *et al*., 2012).

Use of cytogenetic analysis, taxonomy and primate conservation

Primate cytogenetics has shown utility for recognizing species, taxonomy and generally for conservation. Certainly, before you can conserve a species you must be able to identify it. Internationally the species is the biological unit that is protected. We discussed above how the differences in Bornean and Sumatran chromosome 2 suggested, long before it was confirmed by genome sequencing, that these two orangutan populations should be given species status. Chromosome analysis showed that at least three cryptic species existed within the taxon *Galago demidovii* (Stanyon *et al*., 1992). Small ape genera were first recognized on the basis of 2n number (*Hooloch* 38, *Hylobates* 44, *Symphalangus* 50 and *Nomascus* 52) (Prouty *et al*., 1983; Roberto *et al*., 2007; Carbone *et al*., 2012). Subspecies of *Alouatta seniculus* (*sara* and *artoidea*) were show to differ by at least 15 chromosome rearrangements and were certainly two separate species (Stanyon *et al*., 1995; Consigliere *et al*., 1996). Owl monkeys present an impressive example of multiple hidden species. Before the pioneering work of Hershkovitz (1983) only

one species of owl monkey was recognized (*Aotus trivirgatus*) (see Tab. 1). Now at least 11 species are recognized (Hershkovitz 1983; Groves 1993; Menezes *et al.*, 2010; Araujo *et al.*, 2019).

Taxonomic history and diploid numbers of Owl monkeys

Before Hershkovitz	Hershkovitz (1983)	Groves (1993)	Menezes et al. (2010)	2n
Monospecific genus	*A. brumbacki*	*A. brumbacki*	*A. brumbacki*	50
A. trivirgatus	*A. vociferans*	*A. vociferans*	*A. vociferans*	50
	A. lemurinus	*A. lemurinus*	*A. lemurinus*	58
	A. griseimembra	" "	*A. griseimembra*	52, 53, 54
	A. azarae	*A. azari*	*A. azarae*	49, 50
	A. boliviensis	" "	" "	49, 50
	A. miconax	*A. miconax*	*A. miconax*	?
	A. nancymai	*A. nancymaae*	*A. nancymaae*	*54*
	A. nigriceps	*A. nigriceps*	*A. nancymaae*	49,50
		A. trivirgatus	*A. trivirgatus*	*50*
		A. infulatus	*A. infulatus*	49, 50
		A. hershkovitzi	*A. hershkovitzi*	58

Tab. 1. *The history of species recognition and diploid numbers in the New World monkey genus Aotus. The large increase in the number of species from 1 to 11 although extraordinary is paralled by a increase in the number of primate species recognized from less than 200 to over 500.*

An analogous situation was seen in titi monkeys. Some few decades ago a single genus with three species was recognized. Cytogenetic evidence began to accumulate indicating that many more potential species were present Now three genera with 35 species (five new species since 2005) are recognized (Stanyon *et al.*, 2003; Byrne *et al.*, 2016; Araujo *et al.*, 2017).

Considering just owl and titi monkeys there are now 42 additional primates recognized today compared to some 40 years ago. Overall, the number of new primate species recognized has almost tripled in is time period, from less than 200 to more than 500 but, sadly, within 50 years extinction is predicted for a majority of primate species (Estrada *et al.*, 2017).

Certainly, the high number of primate species recognized is correlated factor in the number of species seen veering toward extinction. Certainly, the number of primate species recognized is done so on scientific basis. Traditionally, within the neo-Darwinian context of the synthetic theory of evolution species were recognized

on the basis of reproductive behavior known as the *biological species concept*. This concept held that «species» were not an arbitrary segment of nature's continuum, but real entities that maintain their «realness» because they do not exchange genes.

In the 1980s an alternative concept the *phylogenetic species concept* was introduced. With this definition any population, if it can be distinguished morphologically or genetically from others, warrants being named as a new species. A species is simply the smallest population of organisms measurably different from other populations sharing the same ancestry. Reproductive isolation, or if species hybridize or shares genes, such as between *Homo sapiens* and *H. neanderthalensis* has no implications, is not a criterium for validity of species level distinctions.

The molecular age ushered in numerous instances of apparent cases of hybridization between species which was taken as evidence in support of the phylogenetic species concept as it appeared to less the appeal of the biological species concept. Hybridization was used to explain homoplasy in cytogenetic data as in Cercopithecini monkeys by Dutrillaux *et al.* (1982) The incongruence of mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) phylogenies and chronologies was interpreted as due to between species hybridization. Appeals to hybridization to explain incongruencies between data sets has a fairly long history. Hasegawa *et al.* (1985) to cope with late divergence dates for human-chimp divergence from mtDNA vs nDNA proposed hybridization between humans and chimps after their initial speciation. According to these authors mtDNA was transferred through hybridization between a proto-human and a proto-chimpanzee, after the former had developed bipedalism, probably by the transfer of chimpanzee females to hominid groups. Hybridization between chimpanzee and humans was later used to explain why X-linked and autosomal linked genes gave diverse picture of human origins. According to these authors (Patterson *et al.*, 2006) the human and chimpanzee lineage initially diverged before 6.3 milllion years ago than later hybridized before separating permanently.

More recently widespread hybridization between baboon species was reported (Rogers *et al.*, 2019). But far and away the most widespread use of hybridization to explain genomic data is that applied to evolution of the genus *Homo* and specifically the origins of our own species *Homo sapiens*. Before nuclear DNA sequence data from Neanderthal, researchers insisted that Neanderthal mtDNA was so different that it demonstrated that *H. neanderthal* and *H. sapiens* were separate

species because they had never hybridized (Krings *et al*., 1997). They contrasted the out of Africa model with the multiregional model of modern human origins. In the multi-regional model geographically polytypical population of *H. erectus* adapted to local conditions gene flow blended these population to give origin to *H. sapiens*. Yet no Neanderthal mtDNA was ever found in any population of *H. sapiens*. This supported the view that modern humans evolved in African and in the course of their global migration replaced all previous lineages. Not only did the mtDNA data support the out of Africa model but also strongly supported the biological species concept.

However, once the nuclear genome of Neanderthal was obtained it became clear that Neanderthal shared genes with all non-African modern humans. The explanation was a one-time hybridization between *H. sapiens* and *H. neanderthal* in the middle east when modern humans left Africa and before they migrated over the rest of the globe (Green *et al*., 2010).

Since then there has been a double explosion in both the number of human species and in hybridization events, both between these species and with modern humans. A recent article (Galway-Witham *et al*., 2019) listed the contemporary existence of 9 hominin lineages which overlapped in time with *H. sapiens* with up to 8 lineages of existing contemporaneously with *H. sapiens* (Neandertals, Desinova, archaic Chinese humans, later sunda *H. erectus*, *H. florensis*, *H. lusonesis*). The genomic evidence shows prevalent, multiple episodes of gene flow (hybridization) between these lines and with modern humans (Gokcumen, 2020).

The gap between other human lineages and modern humans appear ever smaller both in genetic and cultural terms. Neandertal behavior appears much similar than previously thought: 1. less clear differences in lithic traditions, 2. presence of distinct cultural groups Neanderthal, 3. construction of shelters, 4. existence of mortuary practices, as well use of varied pigment, jewelry and personal adornments suggest that Neanderthal was a symbolic species, 4. probable use of complex communication systems including language (Galway-Witham *et al*., 2019). It is now thought improbable that cultural formed a barrier between *sapiens* and Neanderthal at least not sufficient to inhibit interbreeding. Indeed, it is now evident that Neanderthal repeatably hybridized with modern humans, there is evidence of at least three separate and varied hybridization with Desinovans. Contrary to previous conclusion, Neanderthal apparently also hybridized with African modern humans (Gokcumen, 2020).

There is also the question of so called «*ghost*», unidentified lineages which contributed genes to modern humans especially in sub-Saharan Africa. However, it may be difficult to distinguish between the contribution of hominin lineages and the deep, still hidden genetic structure of our own species. Some of the populations which held these genes may be now be extinct. Sampling of human populations in some parts of the world, in particular Africa, is still relatively poor and could make hypotheses of archaic contributions and ghost populations a sampling error conclusion.

The finding of a first geneneration offspring between Neandertal and Desinova shows that interbreeding was so prevalent and extensive that it was deemed it an «interbreeding bonanza» (Gokcumen, 2019). Selection of hybridization products may lead to an underestimation of the actual level of interbreeding also raises the possibility that the long-assumed extinction of Neanderthal never really occurred, because the small, isolated remaining groups of Neanderthals were just absorbed by expanding modern humans or that conversely, hybridization can be viewed as an agent of extinction (Wakano *et al*., 2018).

There are now similar examples of hybridization, interbreeding and introgression in non-human primates. Some of these were recently reviewed to provide insights into human evolution (Ackermann *et al*., 2018). There are numerous examples from both New World (marmosets and howler monkeys) Old World monkeys (baboons) and hominoids (Arnold, 2006; Zinner *et al*., 2011; Tung and Barrieos, 2017).

One result of common hybridization for hominins and non-human primates is that the evolutionary tree has become reticulate, taking the form of networks. A braided evolutionary stream is likely a more precise idiographic representation of evolutionary process which effectively invalidates the division of the diverging lines into discrete units or species (Ackermann *et al*., 2018). If we want to conserve the tree metaphor we must reconsider if the various archaic hominins are indeed separate species. Perhaps if we want to consider species as biological units, as in the biological species concept, then the splitting and merging would be considered incomplete speciation. The diverging lines just did not get to the species level distinction.

Of course, the phylogenetic species concept which, does not require rigid reproduction was very welcome by paleontologists. It is near impossible to demonstrate gene flow without accompanying ancient DNA studies on the basis of fossil remains. For the phylogenetics species concept it is enough to recognize *distinguishing* differences. You need only to agree on what these distinguishing differences are which,

may or may not be an easy task. Once gene flow and reproductive isolation were taken away from the definition of species the «objective» basis of species determination was lost. We are left with no way to test whether we have or do not have a species level distinction and the recognition of a species becomes a matter of opinion and authority. Further, the existence of the any particular species becomes extremely difficult to falsify. Without testing and falsification the species becomes an unscientific category and outside of its legal use in conservation efforts has lost value. Since, it is unlikely that the species category will be discarded we urgently need a better definition of what species are. This is no small matter because the definition of species has direct implications for the mode and tempo of evolution that we discussed above. Both species identification and species definition determine how we view the ongoing processes and mechanisms (mode) of evolution. It is fundamental for formulating models of evolution. When every paper published on the genomics of human evolution, and many current papers in primate genomics reveal new episodes or consequences of interbreeding, we can hardly escape the conclusion that hybridization is commonplace. One could argue that multi regional models for our species origins should be reconsidered, but it would be more useful to have new models of human evolution. The development of new models is a work in progress. It will necessitate a through integration of all fields of anthropological knowledge fully justifying the rationale behind the organization of this series of seminars.

Corresponding author: roscoe.stanyon@unifi.it

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Chromosome heteromorphism and karyotype evolution in primates

ORONZO CAPOZZI* MARIANO ROCCHI**
NICOLETTA ARCHIDIACONO** ROSCOE STANYON*

Parole chiave: polimorfismi cromosomici, riarrangiamenti cromosomici, riposizionamento del centromero, citogenetica molecolare.

Riassunto — Studi comparativi di citogenetica molecolare hanno permesso di ricostruire l'evoluzione dei cariotipi dei primati. Tutti i riarrangiamenti cromosomici che caratterizzano le diverse specie hanno obbligatoriamente attraversato un periodo di eterozigosi. Sebbene questo periodo sia in teoria considerato con ogni probabilità breve, la scoperta di eteromorfismi del cariotipo, anche se non del tutto inaspettata, è comunque notevole. Qui riportiamo diversi eteromorfismi recentemente studiati dal nostro gruppo, rimarcando soprattutto gli eteromorfismi sopravvissuti agli eventi di speciazione. In particolare, vengono discussi (i) un polimorfismo di un neocentromero trovato in entrambe le specie di orango. I neocentromeri illustrano l'importanza dell'analisi citogenetica e della mappatura fisica poichè non vengono rilevati dalle tecnologie di sequenziamento. (ii) Eteromorfismi dei gibboni, che erano probabilmente presenti nella popolazione ancestrale. (iii) Una traslocazione Y/autosoma nel presbite argentato che ha causato una rapida evoluzione e una alta variabilità dei cromosomi coinvolti, tanto che ognuno di questi cromosomi riarrangiati sembra una «linea cromosomica» indipendente. Infine (iv) nelle scimmie cercopitecine un eteromorfismo riscontrato in più specie che persiste da almeno 8 milioni di anni, suggerendo che sia stato mantenuto da una specifica pressione evolutiva.

Key words: chromosome polymorphisms, chromosome rearrangements, centromere repositioning, molecular cytogenetics.

Summary — Comparative molecular cytogenetic studies have allowed researchers to tract the evolution of primate karyotypes. Every chromosome rearrangement that characterizes different species obligatorily passed through a period of heterozygosity. Although this period was theoretically considered as brief, the discovery of karyotype heteromorphisms although not totally unexpected are nonetheless notable. Here we report on several heteromorphisms with emphasis on those that have survived speciation events. In particular, we discuss a neocentromere polymorphism found in both species of orangutan. Neocentromeres illustrate the importance of cytogenetic analysis and physical mapping because they go undetected by sequencing technologies. In gibbons heteromorphisms were probably present in the ancestral population and lineage sorting results in a high level of homoplasy in current taxa. A Y/autosomal translocation in the silvered-leaf monkey caused rapid chromosome evolution and variability. Each translocation product appeared to behave as an independent «chromosome lineage». In cercopithecine monkeys a heteromorphism

* Dept. of Biology, University of Florence, Italy.
** Dept. of Biology, University of Bari Aldo Moro, Italy.

found in multiple species lasted at least 8 million years suggesting that it was maintained by positive natural selection for the persistence of heterozygosity.

Introduction

Variability, the basis of evolution, was studied in detail in various organisms at different levels: Single Nucleotide Polymorphisms (SNPs), Copy Number Variation (CNV), and rearrangements of gene order (translocations and inversions). The human genome is the most studied in this respect. Recently, Collins *et al.* (2020) reported on rare Structural Variations (SV), usually more complex than simple inversions and spanning over a megabase, in 3.9% of 14,891 genomes. However, next generation sequence technology used for these kinds of investigations is not fully appropriate to study large rearrangements, especially if they are heterozygous. Indeed, the vast majority of these rearrangements were discovered using classical and molecular cytogenetic methods. The ideal approach is a close integration of two complementary technologies (Rocchi *et al.*, 2006). Indeed, we have contributed our molecular cytogenetic expertise to disclose/validate large cytogenetic rearrangements in the assembly of several primate genomes (Carbone *et al.*, 2014; Gibbs *et al.*, 2007; Locke *et al.*, 2011; Mikkelsen *et al.*, 2005). One relevant category of relatively frequent chromosomal change, centromere repositioning, deserves attention because they were discovered using classical/molecular cytogenetics as it is almost impossible to detect them using sequencing technology (see below).

Karyotype comparison of primates revealed that even closely related species frequently show difference in karyotype organization due to translocations and most frequently, inversions. Each variant chromosome must have originated in a single individual and then spread in the population. Unfortunately, population cytogenetics of primates is poorly investigated. The karyotype of a species is often considered representative of all the individuals of that species even if very few, often only one individual, was characterized. Even for humans, large, cytogenetics-oriented population studies have not been performed. However, prenatal karyotype analysis can be considered as a very large, if unintentional, cytogenetic population study. Examples of centromere repositioning events and inversion, which do not cause phenotypic anomalies, were serendipitously found in these screening (see below). Further, inversions usually do not cause phenotypic

anomalies. Examples are inversions on chromosome 8p (Giglio *et al.*, 2001) and 4p (Giglio *et al.*, 2002) which can reach a relatively high frequencies (up to 39%).

Because population cytogenetics of non-human primates is lacking, our knowledge of primate polymorphisms is poor and surely underestimated. In addition, it is thought that heteromorphisms are usually a temporary situation; eliminated or driven to fixation in a relatively brief time period. Therefore, our recent findings of polymorphisms in primates that have been maintained for long stretches of evolutionary time are particularly noteworthy.

A long-lasting autosomal heterozygosity in Cercopithecini

We recently reported and discuss below a paradigmatic example of heterozygosity in primates that arose at least 8 million years ago and it is maintained in the present-day population of two *Cercopithecus* species: *Cercopithecus albogularis* and *Cercopithecus petaurista* (Tolomeo *et al.*, 2020). It is well known that all the species in the Cercopithecini tribe (genera *Cercopithecus*, *Erythrocebus*, *Chlorocebus*, *Miopithecus*) share a specific chromosome association formed by a fusion of chromosomes homologous to human 20 and 21. This synthetic association can have various forms with different centromere positions among the species belonging to the tribe (Finelli *et al.*, 1999; Moulin *et al.*, 2008; Stanyon *et al.*, 2005; Stanyon *et al.*, 2012). Moreover, in some individuals of *Cercopithecus albogularis* (CAL, Sykes monkey), *C. petaurista* (CPE, lesser spot-nosed monkey), and *C. stampflii* it has been found that the 20/21 chromosome pair can be heterozygous for the centromere position (Lo Bianco *et al.*, 2017; Moulin *et al.*, 2008; Sineo, 1990).

We have characterized the chromosomal organization of the synteny association in two out of three mentioned heterozygous species (*C. albogularis* and *C. petaurista*. Two other Cercopithecini species, *C. aethiops* (CAE) and *E. patas* (EPA), not showing a polymorphism, were used as outgroup (Tolomeo *et al.*, 2020).

Surprisingly, in the analyzed individuals we found five different chromosomal forms that all originated from an ancestral heteromorphism. By reconstructing the flow of rearrangements that generated the variant forms, we disclosed that all of the four analyzed species share an inversion that originated before their separation. Molecular phylogenetic analysis dates the separation of these two groups, CAL-CPE/CAE-EPA, about 8 million years ago (Perelman *et al.*, 2011; Springer *et al.*, 2012), therefore, the chromosomal polymorphism

was maintained for at least this time period.

It is generally believed that inversions are negatively selected because crossing over can generate unbalanced gametes. Therefore, inversion polymorphisms can only be maintained if there is positive selection for the persistence of heterozygosity. Dobzhansky was the first to propose positive selection for the maintenance of inversion polymorphisms in *Drosophila*. He hypothesized that an occasionally created association of favorable coadapted genes was maintained because the inversion suppresses crossing over (Dobzhansky, 1944; Dobzhansky, 1950). More recent works in *Drosophila* provided a strong support for Dobzhansky's hypothesis (Fuller *et al*., 2017; Karageorgiou *et al*., 2019).

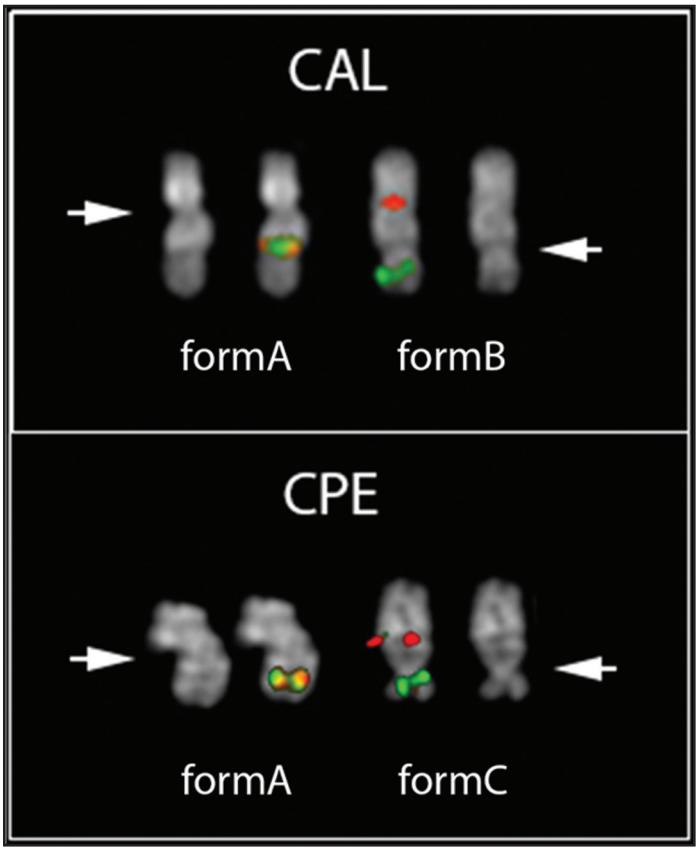

Fig. 1. *FISH results of two BACs (RP11-701C1 in red and RP11-310E20 in green) which flank and partially overlap one of the breakpoints of the first inversion shared by all the variant forms of the Cercopithecini species under study. Forms B and C (formB and formC), which are found in C. albogularis (CAL) and C. petaurista (CPE) respectively, differ in further rearrangements. White arrows indicate the centromere position.*

Heteromorphisms in gibbon karyotypes

Gibbons (hylobatids) show three peculiar, probably interdependent, features: (i) they are, among primates, the most dramatic example of karyotype diversity within a clade; (ii) they experienced an extremely rapid karyotype evolution; (iii) among hominoids this clade has the highest number of species. Rapid karyotype changes may operate as reproductive barriers that facilitates speciation, which result in a high number of species with different karyotypes. Indeed, up to 16 species are currently recognized, divided into 4 genera, each characterized by a different diploid number (*Hoolock* 2n=38, *Hylobates* 2n=44, *Symphalangus* 2n=50 and *Nomascus* 2n=52).

Understanding the chromosome evolution of small apes was particularly challenging. It was clear even from banding analysis alone that karyotype evolution in gibbons was particularly rapid. It was almost impossible to confidently match gibbon chromosome banding with that of other primates and even between small ape genera was very difficult (Stanyon & Chiarelli, 1983). Chromosome painting permitted a higher level of confidence in matching chromosome homologs and confirmed that the small apes had experienced rapid chromosome evolution but did little to resolve phylogenetic and taxonomic relationships (Jauch *et al*., 1992; Koehler *et al*., 1995a; Koehler *et al*., 1995b; Muller *et al*., 2003). To resolve this situation our group published a series of BAC-FISH experiments using arrays of up to 1000 BACs to establish marker order and determine the flow of chromosome rearrangements that occurred during gibbon evolution (Capozzi *et al*., 2012; Carbone *et al*., 2006; Misceo *et al*., 2008; Roberto *et al*., 2007). The paper by Capozzi *et al*. (2012) summaries the BAC-FISH results later confirmed by Carbone *et al*. (2014), who suggested that hylobatids experienced, about 5 million years ago, a near-instantaneous radiation. As a consequence, it is very difficult to establish a linear phylogenetic three of this clade (Capozzi *et al*., 2012; Carbone *et al*., 2014). The hypothesized near-instantaneous radiation strongly suggests the occurrence of multiple incomplete lineage sorting of chromosomal polymorphisms. This hypothesis is indirectly confirmed by the finding of a number of heteromorphisms in the present-day hylobatid populations, although studies in this area are sparse.

Even from chromosome banding there was indications that gibbon species could be polymorphic for variant chromosome forms. Stanyon *et al*. (1987) analyzed 27 gibbons from the genus Hylobates (*H. lar, H. agilis, H. klossi, H. muelleri, H. moloch, H. pileatus*). All species had nearly

identical banded karyotypes but three forms of chromosome 8 were found that apparently differed for pericentric inversions: four of the six taxa had polymorphic individuals (Van Tuinen *et al.*, 1999). Later, with chromosome painting, Hirai showed that a translocation between chromosomes 8 and 9 was also involved (Hirai *et al.*, 2003).

Variant forms of chromosomes 4 and 5 exist in the *Symphalangus syndactylus* (Capozzi *et al.*, 2012). In *Nomascus leucogenys* (NLE) an inversion on chromosome 7 generated the NLE7b form. In this same species a translocation between chromosomes NLE1 and NLE22 generated variants 1b and 22b chromosomes (Couturier & Lernould, 1991; Koehler *et al.*, 1995b).

The analysis of Capozzi *et al.* (2012) suggested that the homoplastic distribution of chromosome forms in Hylobatidae could be best explained if the ancestral species for all living small apes were polymorphic for various chromosomes. Present day distributions would be explained by incomplete lineage sorting due to demographic parameters and hybridization and introgression between diverging evolutionary lines.

Rapid evolution of heteromorphic sex chromosomes

The sex determination system, consisting of two heteromorphic (X and Y) chromosomes, is highly conserved among placental mammals. Comparative studies have shown that X chromosome rearrangements are very rare during evolution (Kim *et al.*, 2017). The reason for this is explained by X chromosome inactivation in females (Lyon hypothesis). A simplified explanation of an actually complex phenomenon is that in XX female mammals, early in embryonic development, one X in each cell is randomly inactivated. The random inactivation, clonally inherited, lead to a mosaicism in which defective recessive genes in heterozygous state are compensated by the normal counterpart active in approximately half of the cell population.

Probably because of the peculiar functional gene balance of the X chromosome, X/autosome translocations which were fixed in a primate population have never been found. And even a single individuals with such translocation have been never described in non-human primates even if in humans due to large scale screening some cases of X/autosome translocation were found. The absence of X/autosome translocations in nonhuman primates strongly suggest that these translocations have no evolutionary perspective.

The Y chromosome is mainly composed of heterochromatin,

harbors very few genes, and is extremely variable in morphology both within and between species. The two sex chromosomes pair in meiosis, pairing being crucial for correct chromosome segregation, but it is limited to a short segment, called Pseudo Autosomal Region (PAR), located on the tip of both X and Y chromosomes.

Contrary to the X chromosome, some cases of Y/autosome translocation have been observed in in both OWMs (Stanyon *et al.*, 2011) and NWMs (Araújo *et al.*, 2017; Bigoni *et al.*, 1997; Consigliere *et al.*, 1996; Margulis *et al.*, 1995; Steinberg *et al.*, 2017). In a recent work we have reconsidered and characterized in detail the Y/autosome translocation in silvered-leaf monkey (*Trachypithecus cristatus*, TCR), the only rearrangement of this type known among the catarrhine monkeys (Capozzi *et al.*, 2018). Due to a translocation between Y and chromosome 1 (human 5), a system of sex chromosomes is X1X2 / Y1Y2 was generated. Note that sex chromosomes were considered as all the chromosomes which were unpaired in the male. X1 equals the normal X chromosome, X2 equals TCR1; Y1 and Y2 equal the reciprocal translocation products t(1;Y). However, the reason why this example is under consideration here, lies in the fact that the karyotype analysis of different TCR individuals showed that chromosome 1 was present in at least three different forms (Bigoni *et al.*, 1997) which were extremely and independently rearranged, in contrast to the high level of marker order conservation of the other silvered-leaf monkey chromosomes. Each translocation product appeared to behave as an independent «chromosome lineage». We were able to reconstruct the evolutionary history of these «lineages» by comparing them to their homologous chromosomes in two other colobine species: the African mantled guereza (*Colobus guereza*) and the Indian langur (*Semnopithecus entellus*). The most peculiar result of the work was the finding of a massive reuse of breakpoints: only 12, out of the 40 breaks we characterized, occurred in domains never reused in other rearrangements. Some domains were used up to four times.

Unlike the karyotype of the gibbons, where all the chromosomes have undergone a high number of rearrangements, in this case only the chromosomes involved in the translocation have had a rapid evolutionary path. We interpreted these data assuming that the barrier to recombination caused by the translocation accelerated the evolutionary process of each of these chromosomes.

Chromosome polymorphisms and Centromere repositioning

In 1976 Seuanez *et al.* (Seuanez *et al*., 1976) found a polymorphism of orangutan chromosome 9 (homolog to human 12) in both orangutan species (Borneo and Sumatra), illustrating how a chromosome polymorphism could be maintained over time and even survive speciation events. It also raised the intriguing question of whether polymorphism could be maintained by positive selection. Originally this polymorphism was thought to be complex rearrangement but was later shown to be a centromere repositioning event (Locke *et al*., 2011; Rocchi *et al*., 2012).

Centromere repositioning is when the centromere moves from its original position to a different location along the chromosome without any other structural rearrangements such as inversions. The sequence domain of the newly seeded neocentromere is usually indistinguishable from the normal sequence counterpart on the homologous chromosome. This feature makes the newly formed centromere completely undetectable by sequencing.

The phenomenon was discovered because, in some primates, the position of the centromere had an evolutionary history independent from the surrounding markers. These centromeres, coined as Evolutionary New Centromeres (ENC), are relatively frequent (Rocchi *et al*., 2012) and in macaques, 9 out of 22 chromosomes shifted centromere over evolutionary time (Ventura *et al*., 2007). The ENC present in orangutan is a paradigmatic example.

Analysis of the neocentromere in the orangutan showed that there was no change in marker order and therefore no structural rearrangements between the two homologs. In a sample of over 120 orangutans the frequency of neocentromere was about 25% in both Sumatran and Bornean orangutans (de Boer & Seuanez, 1982; Rocchi *et al*., 2012). Later, sequencing of orangutan neocentromere chromosome domain and comparison with the wild counterpart showed, despite its age (at least 400,000 years (Locke *et al*., 2011)) no differences in sequence, therefore supporting that no DNA sequence codes for centromere formation and that centromeres form epigenetically (Tolomeo *et al*., 2017). The sliding of a neocentromere in horse further confirmed the sequence-independence, epigenetic nature of neocentromeres (Purgato *et al*., 2015). The analysis of ENC of much older age, as those reported in macaque (Ventura *et al*., 2007), showed that, over time, the centromere acquires the complex structure characteristic of normal centromeres. The discovery of evolutionary new centromeres in the previous decade

had already falsified hypothesis of centromere conservation and introduced a new mode of chromosome evolution to genomic studies.

Neocentromeres in evolution and disease

The centromere, (Darlington, 1936) is the primary constriction where the kinetochore forms to insure correct division of hereditary material in cell division (meiosis and mitosis). The centromere is a sequencing black hole almost composed of specific repeat arrays and frequently surrounded by other satellite DNAs. In primate centromeres large arrays of 171 bp alpha satellite units compose its core.

Starting some 25 years ago many neocentromeres have been reported, in humans and in other organisms. Neocentromeres are essentially «nude» and have none of the repeats and satellite DNA found in «normal» centromeres. In humans they were discovered because they usually stabilize an extra chromosomal fragment which causes phenotypic anomalies (coined «clinical» neocentromeres). Sequence data available for some of these neocentromeres failed to show any shared sequence feature(s) that could predict a potential capability to form neocentromeres, further supporting the conclusions reported above. In contrast to the underlining DNA variability surrounding normal centromeres, the proteins responsible of the centromeric function are highly conserved.

Many human neocentromeres have been reported in medical literature (Marshall *et al*., 2008) with four main clusters. Recently, researchers have shown that the domains where human «clinical» neocentromeres were formed had occasionally a very interesting evolutionary history. As an example, is the cluster mapping to human chromosomes 15q24-26. From evolutionary genome studies it is certain that chromosome 14 and 15 formed a very ancient mammalian chromosome, which was fissioned in the hominoid ancestor (Stanyon *et al*., 2008; Ventura *et al*., 2003). The fission generated two chromosomes corresponding, in humans, to chromosome 14 and 15. As shown in the Fig. 2, two neocentromeres formed stabilizing the two resulting chromosomes, while the old centromere (at 15q25) was inactivated. Clusters of segmental duplications, typically surrounding a mature mammalian centromere, represent the remains of the inactivated ancestral centromere, and, very interestingly, this is exactly the region where numerous clinical neocentromeres formed (Ventura *et al*., 2003).

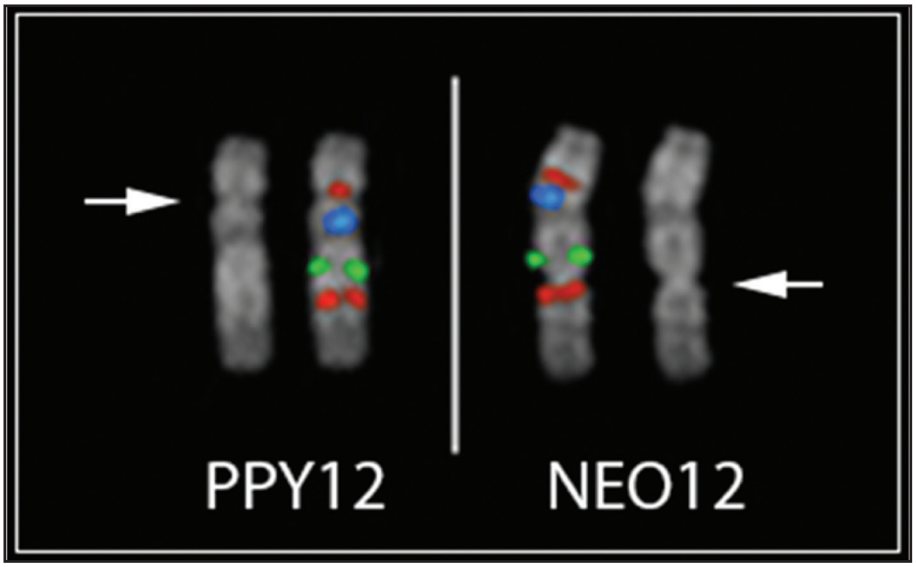

Fig. 2. *FISH results on orang chromosome 12 show the identical order of four sequentially arranged BAC probes indicated in red (RP11-12I7 and RP11-20L19), blue (RP11-80I23) and green (RP11-10O10) between the normal (PPY12) and the neocentromere (NEO12). The outermost chromosomes in the image are only DAPI images that show the primary constriction of both chromosomal forms, indicated by arrows. The neocentromere-bearing variant is polymorphic in both Bornean and Sumatran populations, suggesting the neocentromere arose before the Bornean/Sumatran split.*

Human evolutionary neocentromeres

We have seen that ENC are relatively frequent. We can assume, however, that not all the ENC events occurring in a population will be fixed or at least spread in that population. The discovery of the actual frequency of such events would imply extreme large molecular cytogenetic population studies. This kind of studies are clearly impracticable in nonhuman primates, but, again, prenatal diagnoses practice comes to our aid again. Indeed, as far as we know, at least 8 cases of centromere repositioning events have been published (Liehr *et al.*, 2010). One in particular, seeded at 6p22.1 is of notice. It was a familial case, found through a prenatal diagnosis, we described few year ago (Capozzi *et al.*, 2009). The evolution of chromosome 6 in primates showed that the normal human centromere originated recently. It was previously located at 6p22.1, in the same chromosomal domain where our case was seeded, i.e. it jumped back to the position it had about 17 million years ago.

Conclusions

In this short review we have discussed a number of cases of chromosomal heteromorphisms and polymorphisms in primates. Given that population studies of primate species are rare, usually only a few, even one individual per species has been reported, it is hard to escape the conclusion that chromosome heteromorphisms and true polymorphism (at least 1% frequency) are much more common than generally appreciated. It is now clear that polymorphisms can be maintained for long stretches of evolutionary time, literally millions of years, survive speciation events and may be maintained by a positive perhaps even balancing selection. It is also probable that polymorphisms in ancestral populations combined with demographic parameter promoting lineage sorting can compound problems using chromosomes for phylogenomic analysis. Finally, it is instructive that the evolutionary processes and history of chromosomes can be informative about contemporary, even clinical genomic phenomena. The relationships between neocentromeres in evolution and clinical genetics shows how evolution provides a convincing explanation for contemporary genomic phenomena of medical interest. Dobzhansky's famous sentence is very truly appropriate here: «Nothing in biology makes sense except in the light of evolution». Finally, much research remains to be done especially integrating these intriguing results at the cytogenetic level with sequencing efforts to more fully resolve the interaction of chromosome rearrangements and the evolution of species.

Corresponding author: roscoe.stanyon@unifi.it

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Complete mitochondrial genome of *Macaca mulatta* from Bangladesh

KAZUNARI MATSUDAIRA* TAKAFUMI ISHIDA*

Parole chiave: macaca rhesus, Bangladesh, Cina, India.

Riassunto — *Macaca mulatta* (macaca rhesus) ha un range di ampia distribuzione tra i primati non-umani. Mentre molti studi genetici sono stati condotti su macache rhesus provenienti da India e Cina, gli studi sulle macache rhesus nel centro della distribuzione della specie sono relativamente limitati. In questo articolo riportiamo la intera sequenza di genoma mitocondriale di una macaca rhesus proveniente dal Bangladesh. L'analisi filogenetica riveìa che la macaca rhesus dal Bangladesh è più strettamente imparentata con le macache della Cina occidentale che con la maggioranza delle macache rhesus della Cina orientale e dell'India, almeno per quanto riguarda il loro genoma mitocondriale.

Key words: rhesus macaque, Bangladesh, China, India.

Summary — *Macaca mulatta* (rhesus macaque) has a large distribution range among non-human primates. While many genetic studies have been conducted for rhesus macaques originating from India and China, studies on rhesus macaques in the center of the species distribution are relatively limited. Here, we report on the whole mitochondrial genome sequence of a rhesus macaque from Bangladesh. Phylogenetic analyses revealed that the Bangladeshi rhesus macaque is more closely related to western Chinese than the majority of Indian and eastern Chinese rhesus macaques at least in respect to their mitochondrial genome.

Introduction

Among non-human primate species, *Macaca mulatta* (rhesus macaque) has the largest geographic distribution, which includes Afghanistan, Pakistan, India, Nepal, Bhutan, Bangladesh, China, Myanmar, Thailand, Laos, and Vietnam (Fooden, 2000). Rhesus macaques are among the most important biomedical model species. Genome-scale studies have focused on the rhesus macaques of Indian and Chinese origins (Hernandez *et al*., 2007; Liu *et al*., 2018; Xue *et al*., 2016). These studies have uncovered their evolutionary histories. Unfortunately, studies on rhesus macaques from other areas are limited to only partial mitochondrial DNA (mtDNA) analyses.

Phylogenetic studies on the hypervariable region 1 (HVR1) of the

* Department of Biological Sciences, School of Science, The University of Tokyo.

mtDNA suggested that Bangladeshi, Myanmar and a small portion of Indian (Ind2) rhesus macaques form a mtDNA clade which is different from the majority of Indian (Ind1) and Chinese rhesus macaques (Hasan *et al*., 2014; Smith and McDonough, 2005). However, due to the short length of the fragments analyzed phylogenetic relationships with the Ind1 and several Chinese mtDNA clades was not resolved. To obtain a clearer mitochondrial phylogeny and divergence time between rhesus macaques, we determined the whole mitochondrial genome (mitogenome) sequence of a rhesus macaque from Bangladesh and compared the sequence with the rhesus mitogenome sequences available in GenBank.

Materials and Methods

DNA was extracted from a dried residue of plasma of a rhesus macaque sampled in East Pakistan (current Bangladesh) by a Japan-US collaboration project (Ishimoto *et al*., 1970). We dissolved the residue in 180 μL deionized water and extracted DNA by using the QIAamp DNA Mini Kit (Qiagen, Germany). We conducted PCR following the methods reported in a previous study (Matsudaira *et al*., 2018). Two consecutive PCRs were done to avoid the amplification of the nuclear insertions of mtDNA. We then purified PCR products by using the FavorPrepTM PCR Clean-Up Mini Kit (Favorgen, Taiwan) and outsourced Sanger sequencing to FASMAC (Japan). The first set of PCRs was conducted for three overlapping fragments.

Primers were:

5′-GACCGTGCAAAGGTAGCATAATC-3′ and
5′-GATGTGTCTAACTGGGGCATTTC-3′ for fragment 1;
5′-TTCCCACACTAGGCCTAAAAACA-3′ and
5′- CGTATCCTCCTCAGATTCATTGG-3′ for fragment 2;
5′-CTGGGGGCTATTACTACCCTATTT-3′ and
5′-AGTGGGCCTTATTTCTCTTGTCC-3′ for fragment 3, respectively.

The second set of PCRs was conducted for 29 overlapping fragments using the first PCR products as a template. Primer sequences for the second PCR will be provided by authors upon request.

We determined the 16,582 bp mitogenome sequence consisting of 13 protein-coding genes, two ribosomal-RNA, and 22 transfer-RNA. The mitogenome sequence was deposited in DDBJ/EMBL/GenBank with Accession No. LC496787. There were seven rhesus mitogenomes

deposited in GenBank (Accession Nos. AY612638, JQ821843, KF830702, KJ567051, KJ567053, KP641672, and KX401548). Of those, detailed origins were known for two: KP641672 from Hainan Island (Liu *et al*., 2016) and KX401548 from Tibet (Su *et al*., 2019). Two other sequences, KF830702 and KJ567051, were from China (Liedigk *et al*., 2014; Wu *et al*., 2016) and one, KJ567053, was from India (Liedigk *et al*., 2014), respectively. Origins of the other two sequences were not reported. Prior to mitogenome phylogenetic analysis, clade affinities of the eight rhesus mitogenomes were confirmed by a phylogenetic analysis of HVR1 sequences obtained from GenBank. The dataset included 651-bp sequences of 610 rhesus, 38 Japanese (*Macaca fuscata*) and 54 Taiwanese (*Macaca cyclopis*) macaques. A neighbor-joining tree was constructed based on the Tamura-Nei model with 1,000 bootstrap resampling by using MEGA6 (Tamura *et al*., 2013). Our rhesus mitogenome tightly clustered with the partial mtDNA of Bangladeshi rhesus macaques (data not shown). In addition, this clade also included mtDNA of Myanmar, Thai and Ind2 rhesus macaques. The seven other rhesus mitogenomes represented other major rhesus mtDNA clades. AY612638, JQ821843 and KJ567051 represented Ind1 with Nepali rhesus clade. KF830702, KJ567051, and KP641672 (Hainan Island) represented eastern Chinese with Vietnamese rhesus clade. KX401548 (Tibet) represented western Chinese rhesus clade. In the HVR1 tree, the relationship among the four major clades was not well resolved.

For mitogenome phylogenetic analyses, we included the eight rhesus mitogenomes and seven mitogenomes of five other macaque species with one mitogenome of Anubis baboon (*Papio anubis*). We focused on the 13 protein-coding genes and two ribosomal RNA, and the dataset consisted of 13,844 bases. Maximum likelihood (ML) tree was constructed by using IQ-TREE (Nguyen *et al*., 2015) with substitution model and partition scheme selection by ModelFinder (Kalyaanamoorthy *et al*., 2017), which detected three partitions among the 15 regions: partition 1 (ATP6, ATP8, COX2, CYTB, ND1, ND2, ND4, ND5, ND6) with TN+F+G4 model, partition 2 (COX1, COX3, ND3, ND4L) with TN+F+G4 model, and partition 3 (12SrRNA, 16SrRAN) with TIM2+F+R2 model. An Ultrafast Bootstrap Approximation test (Minh *et al*., 2013) was performed with 1,000 resampling iterations. Divergence time was estimated by using BEAST 2.5 (Bouckaert *et al*., 2019). In the Bayesian analysis, we used a relaxed log-normal clock model and a birth-death tree prior model. The divergence time prior for Barbary macaques (*Macaca sylvanus*) and other macaque species was set as a normal distribution prior with a mean of 5.5 million years

ago (mya) and sigma of 0.51 (i.e. 4.5-6.5 mya for 95% credibility interval [CI]) (Alba *et al.*, 2014). We used the same partition scheme and models in the ML analysis except using GTR+F+G4 model for the partition 3. Two independent runs including 40,000,000 Markov chain Monte Carlo generations were performed, and parameters were sampled once per 2,000 generations. The convergence of the parameters was checked with Tracer 1.7 (Rambaut *et al.*, 2018). The first 25% samples of each run were discarded as burn-in, and thus 30,002 tree parameters were merged and used for making the consensus tree by LogCombiner and TreeAnnotator of BEAST 2.5. The tree was visualized with FigTree 1.4.4 (https://github.com/rambaut/figtree/releases).

Results and Discussion

The ML and the Bayesian analyses resulted in the same tree topology with high statistical supports for all the nodes (Fig. 1).

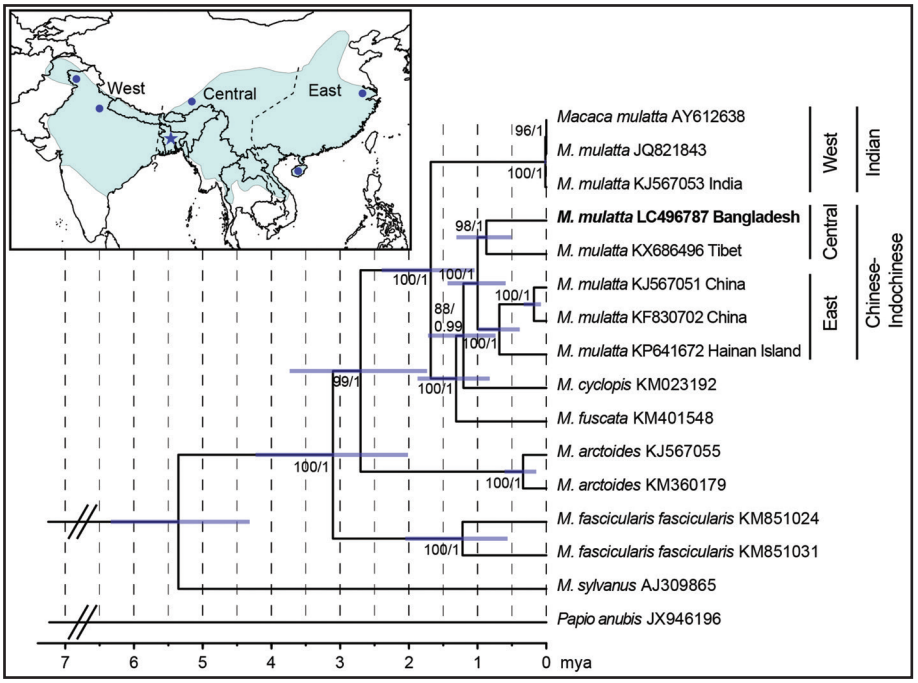

Fig. 1. *Approximate sampling locations and whole mitochondrial genome phylogenetic tree with divergence time of rhesus macaques. The distribution range was redrawn based on Fooden (2000). The values on the nodes are bootstrap values of IQTREE analysis and posterior probabilities of BEAST analysis. The bars on the nodes indicate the 95% credibility interval of divergence time.*

In the tree, Ind1 clade («West» in Fig. 1) consisting of three rhesus mitogenomes located at the basal position among rhesus/Japanese/Taiwanese macaques and diverged from the others 1.68 mya (1.04-2.40 mya; 95% CI). Then, Japanese and Taiwanese macaque mitogenomes diverged consecutively from the other five rhesus mitogenomes at 1.31 mya (0.82-1.87) and 1.21 mya (0.74-1.72), respectively. Among the remaining five rhesus mitogenomes, Bangladeshi rhesus mitogenome clustered together with Tibetan rhesus mitogenome (western Chinese clade) («Central») and diverged from eastern Chinese rhesus mitogenome («East») around 1.00 mya (0.59-1.44). The divergence between Bangladeshi and Tibetan rhesus mitogenomes was estimated to occur at 0.87 mya (0.51-1.31).

Our mitogenome analysis successfully resolved the phylogenetic relationships of major rhesus mtDNA clades, which was unattainable in the previous partial mtDNA studies (Hasan *et al*., 2014; Smith and McDonough, 2005; Tosi *et al*., 2003). The close relationship between Bangladeshi (which clustered with Myanmar, Thai and Ind2 rhesus mtDNA in HVR1) and western Chinese clades is consistent with the previous study using a set of autosomal microsatellites that suggested a smaller genetic divergence between Myanmar and Chinese rhesus macaques than either between Myanmar and Indian or Chinese and Indian rhesus macaques (Kanthaswamy *et al*., 2008).

Because east-west divergence of Chinese clades occurred prior to the divergence of Bangladeshi and western Chinese clades, the expansion of the distribution might have occurred west-southward from western China to Bangladesh rather than east-northward from Bangladesh to China. This result supports a previous hypothesis for the westward expansion of rhesus monkeys in Bangladesh (Hasan *et al*., 2014). The common ancestor of the «Central» and «East» clades may have originated around Tibet to Yunnan where western Chinese clade currently occupies (Su *et al*., 2019). The mtDNA divergence among all the Chinese and Bangladeshi rhesus clades, that was estimated to have occurred about 1.00 mya (0.59-1.44), might reflect the expansion of Chinese rhesus populations before the Xixiabangma Ice Age (0.80-1.17 mya) and then the reduction (and probably isolation) of the populations during the glacial period detected by a genome study (Liu *et al*., 2018).

Since gene flow in female-philopatric macaques is mainly mediated by migrating males, mitogenome analysis is one-sided (Melnick and Hoelzer, 1992) and thus a possible gene flow between Bangladeshi and Indian rhesus macaques granting genomic makeup of Bangladeshi rhesus differentiated from that of Chinese or even from Myanmar

rhesus macaques is postulated. Further genome-scale studies including rhesus macaques from Bangladesh, northeast India, Myanmar, and Thailand are essential to uncover more details of the rhesus macaque genomic diversity. The expected results shouldshed light on their geographic expansion and divergence timeframe of *Macaca mulatta*.

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Corresponding author: Kazunari Matsudaira: kaz.matsudaira@gmail.com

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The origin and history of native Japanese chickens based on the mitochondrial DNA

TAKAHIRO YONEZAWA* MASAOKI TSUDZUKI**
YASUTAKA IMAMURA*** MASAHARU MATSUZAKI****
SHOGO MATSUNAGA***** SATORU FUKAGAWA*****
HIROSHI OGAWA* TAKESHI SASAKI*
FUMIHITO AKISHINONOMIYA****** YOSHIO YAMAMOTO**

Parole chiave: sequenze mtD-loop, diversità, domesticazione animale.

Riassunto — Nonostante l'alto livello di diversità nelle razze native di polli presenti in Giappone, le loro origini e storie sono poco conosciute. Studi precedenti basati su evidenze morfologiche e di archivio suggeriscono che le razze di pollo giapponese (JNC) abbiano avuto origine da razze diffuse in Giappone e identificate con i nomi «Jidori», «Shokoku», and «Shamo». Il termine Jidori non si riferisce in realtà a razze, ma è un nome generico di polli locali che conservano caratteri primitivi visti nel «red junglefowl», e che si pensa siano stati introdotti in Giappone durante l'Era Yayoi via Corea. Shokoku è una bella razza che si ritiene sia stata importata dalla Cina nell' Era Heian. Shamo è una razza specializzata nella lotta e si dice sia arrivata dalla Tailandia durante il periodo Edo. Tuttavia questa ipotesi non è stata esaminata da test molecolare. In questo studio abbiamo determinato la completa sequenza mitocondriale D-loop di 13 razze JNC con 149 individui, e condotto analisi genetiche popolazionali e filogeografiche combinate con le sequenze D-loop sequences di 5.563 polli asiatici riportate in precedenti lavori. I risultati delle nostre analisi suggeriscono che le componenti genetiche di JNC sono essenzialmente vicine a quelle delle regioni nordiche dell'Asia orientale.
Le razze Jidori si separarono in quattro gruppi suggerendo molteplici origini ed eventi indipendenti di diffusione in Giappone probabilmente da Cina e Corea.
Le componenti genetiche di Shokoku e Shamo sono vicine a quelle dei polli del nord-est asiatico. D'altra parte parecchie razze derivate da Shokoku e Shamo hanno simili componenti genetiche con i gruppi Jidori, suggerendo che queste razze furono stabilite per mezzo di crossbreeding con Jidori. La componente genetica di Shamo contraddice la convinzione generale che la loro origine di provenienza sia il sud-est asiatico. Tuttavia, poichè Shamo possiede anche aplogruppi mitocondriali specifici dal sud-est asiatico al sud della Cina, è possibile che Shamo si sia stabilito con una struttura genetica multi-strato.

Key words: mtD-loop sequences, fowl diversity hotspot, animal domestication.

Summary — In spite of the high diversity of the native chicken breeds established in Japan, their origins and histories are little known. Previous study based on the

* Faculty of Agriculture, Tokyo University of Agriculture.
** Graduate School of Integrated Sciences for Life, Hiroshima University Kagamiyama.
*** Kumamoto prefectural Uki public health center.
**** Higo Chabo Club.
***** Nagasaki Agriculture and Forestry Technical Development Center.
****** The University Museum, The University of Tokyo.

morphological and archival evidence suggested native Japanese chicken (JNC) breeds were established based on three breeds namely «Jidori», «Shokoku», and «Shamo» propagated to Japan. Jidori are actually not breeds, but the generic name of local chickens that retain primitive characters seen in the red junglefowl and thought to be introduced to Japan in Yayoi Era via Korea. Shokoku is an aesthetic breed that is believed to have been imported from China in the Heian Era. Shamo is a specialized breed for cockfighting and is believed have come from Thailand during the Edo Era. However, these hypotheses have not been examined by the molecular evidence. Here, we determined the complete mitochondrial D-loop sequence of 13 JNC breeds in 149 individuals, and conducted phylogeographic and population genetic analyses together with D-loop sequences of 5,563 Asian chickens reported in previous publications. The results of our analyses suggested that the genetic components of JNC are essentially close to those of north regions of East Asia. Jidori breeds were separated into four groups suggesting their multiple origins and independent propagation events to Japan probably from China and Korea. Genetic components of Shokoku and Shamo are also close to those of north East Asian chickens. On the other hand, several breeds derived from Shokoku and Shamo have similar genetic components with Jidori groups, suggesting these breeds were established by the crossbreeding with Jidori. The genetic component of Shamo is contradictory with their origin generally believed to be from Southeast Asia. However, since Shamo also possesses mitochondrial haplogroups specific to Southeast Asia to South China, it is possible that Shamo has been established with a multi-layered genetical structure.

Introduction

Currently about 200-400 breeds of domestic chickens (*Gallus gallus domesticus*) are recognized, and about 40 native breeds among them have been established in Japan (Tsudzuki, 2003). Various breeds of native Japanese chickens (JNC) were developed not only for food, but also for socio-cultural purposes such as games, aesthetics, and crowing. Japan is one of the global diversity hotspots of chicken breeds. However, the origin and breeding history of JNC, especially the breeds established before the Meiji Era (1868-1912), are still controversial.

Oana (1951) proposed a hypothesis on their origin and history based on morphologies and archival records, which suggested that JNC were mainly established based from three breeds, namely «Jidori», «Shokoku», and «Shamo (Ô-Shamo)». These breeds were independently introduced from foreign countries in different periods. Jidori are local chickens that retain primitive characters seen in the red junglefowl (*G. gallus*), the wild progenitor of domestic chickens. Oana (1951) suggested that the ancestor of Jidori was introduced to Japan in the Yayoi Era (1,000 BC-300 AD) via the Korean Peninsula. Jidori is actually a generic name not specifying a breed. Jidori is now classified into

several modern breeds such as Tosa-Jidori, Ise (Mie)-Jidori, Gifu-Jidori, and Iwate-Jidori depending on their localities. Shokoku is an aesthetic breed. It is believed that it was imported from China in the Heian Era (794-1,185), and several additional breeds such as Onagadori (long tailed chicken) and Totenko (long clawing chicken) were derived from Shokoku (Oana, 1951). Shamo is a breed specialized for cockfighting and is believed have come from Thailand during the Edo Era (1,603-1,868). Currently, several varieties of Shamo are recognized. These varieties and the dwarf fighting cock, called Ko-Shamo are thought to be directly derived from Shamo. According to Oana (1951), many JNC breeds were established as direct derivatives from different Jidori lineages, Shokoku lineage, and Shamo lineage, or by crossbreeding of these lineages in the Edo Era. In addition, several other breeds such as Japanese bantam called «Chabo», White Silkie called «Ukokkei», and the extinct large sized black colored chicken called «Dai-Tomaru» were introduced from East and Southeast Asian countries by the Edo Era. New breeds, such as Nagoya, Tosa-Kukin, Kumamoto which were mainly developed for the food productivity by crossbreeding with the Western commercial breeds were developed during or after the Meiji Era.

Oana (1951)'s hypothesis is simple, attractive, and gains support form genetic studies based on the blood types. The blood protein polymorphisms demonstrated that the JNC breeds can be roughly separated into three lineages represented by Jidori, Shokoku, and Shamo (Okada *et al.*, 1984, Hashiguchi *et al.*, 1984). However, other studies with different taxon schemes and protein loci (e.g., Okabayashi *et al.*, 1998) yielded different, alternative phylogenetic relationships among JNC. Unfortunately, Oana's (1951) evolutionary scenario has not yet been extensively tested using the molecular genetic evidence. Osman *et al.* (2006) analyzed the phylogenetic relationships among JNC breeds based 20 microsatellite loci and indicated that Shokoku and its related breeds formed a clade, but Shamo and its related breeds as well as Jidori do not form clades. Moreover, Osman *et al.* (2006) used only two commercial breeds to represent foreign breeds. These are too few to establish secure geographical origins of JNC which therefore remains an enigmatic issue.

Komiyama *et al.* (2003, 2004) used mtDNA (mitochondrial DNA) to analyze JNC breeds together with the red junglefowls. They concluded that all JNC including aesthetic and long-crowing breeds were derived from Shamo via Ryukyu, the southwestern islands of Japan. However, since Komiyama *et al.* (2003, 2004) mainly used Shamo and its related

breeds for their phylogenetic analysis, their conclusion seems to be largely biased by the taxon sampling. Later Oka *et al.* (2007) extensively sampled various JNC breeds, carried out a phylogenetic and population genetic analyses. They concluded that the JNC can be separated into a Jidori-Shokoku related group and a Shamo related group. They further concluded that both groups were independently introduced from China and Korea and from Southeast Asia. However, even after the publication of Oka *et al.* (2007), mtDNA data from the domestic chickens and the red junglefowls were still limited at the global level (but see also Liu *et al.*, 2006). Oka *et al.* (2007) also failed to elucidate the origins and histories of JNC breeds.

To help remedy this situation, Miao *et al.* (2013) exhaustively collected all available mtDNA data from public database and defined the haplogroups together with their own 2,093 of *de novo* sequences. They further demonstrated the regional differences of the haplogroup composition in the world. Miao *et al.* (2013)'s work was recently updated by Huang *et al.* (2018). Although mtDNA analysis is limited to information concerning maternal lineages, it still can provide valuable information on the origins and histories of the JNC breeds and provide the basis for extensive comparisons of mtDNA of the JNC breeds with those of globally sampled chickens. With the aim of addressing these issues, we newly determine the complete mtDNA D-loop sequences from the JNC mainly from Jidori and compared these and published JNC data with the data of chicken mainly collected from various Asian countries.

Materials and Methods

Samples

In this study, 149 individuals from 13 JNC breeds were analyzed. Whole blood samples of Ise-Jidori (16 individuals), Ryujin-Jidori (18 individuals), Iwate-Jidori (26 individuals), and Gifu-Jidori (8 individuals) were collected from Japanese Avian Bioresource Project Research Center, Hiroshima University. Whole blood samples of Tsushima-Jidori (32 individuals) were collected from Nagasaki Agriculture and Forestry Technical Development Center. Whole blood samples of Kurekodori (8 individuals), Taikan-Katsura-Chabo (6 individuals), Daruma-Chabo (3 individulas), and Jisuri (4 individuals) were provided from the members of Higo Cabo Club as well as Kumamoto Prefectural Kikuchi Agricultural High School and Kumamoto City Zoological and Botanical

Gardens. Purified DNA samples of Tokuji-Jidori (6 individuals), Sadohige-Jidori (2 individuals), Aizu-Jidori (10 individuals), and Tosa-Jidori (10 indivduals) were provided from FASDA (The Foundation for Academic Specimens of Domesticated Animals). Whole blood samples and purified DNA samples were stored in -20°C and -80°C until used for the experimental works.

DNA extraction, PCR, and Sequencing

Whole genomic DNA was purified from the whole blood samples by the standard phenol–chloroform method (Green and Sambrook, 2012). The mitochondrial complete D-loop sequence of JNC were amplified by the PCR method and determined by the direct sequence using 3500xL Genetic Analyzer (Applied Biosystems). The detailed procedures were followed according to Osman and Nishibori (2014). Nucleotide sequence data determined in this study were deposited in DDBJ under the accession numbers of LC586649-LC586797.

Phylogenetic and Population Genetic Analyses

The complete D-loop sequences of the JNC by Oka *et al.* (2007) and Osman *et al.* (in press) were retrieved from NCBI. Since Oka *et al.* (2007) registered one sequence for each haplotype in NCBI, the multi-fasta file with the reconstructed haplotype frequencies for each breed were manually generated, and aligned automatically by the MAFFT program ver. 7.428 (Rozewicki *et al.*, 2019) together with the new sequence data of JNC by this study. This data alignment consists of 295 individuals from 30 breeds with the length of 1,232 bp. The evolutionary relationships among breeds were estimated by the following procedures: the net genetic distances between breeds were estimated using MEGA 7 (Kumar *et al.*, 2016) with the correction by the TN93 (Tamura and Nei 1993) model. If there are negative values in the net genetic distances, they were replaced to 0. The *Fst* values between breeds was estimated by DNASP program ver. 6.12.03 (Rozas *et al.*, 2017).

Based the net genetic distance and *Fst* matrices, the Neighbor Networks (Huson and Bryant, 2006) were estimated by the SplitsTree program ver. 5 (https://software-ab.informatik.uni-tuebingen.de/download/splitstree5/welcome.html).

Subsequently, the domestic chickens in East Asian, Southeast Asian, South Asian, and Pacific (including Indonesia) countries published in previous works (see Miao *et al.*, 2013; Xiang *et al.*, 2014; Huang *et al.*, 2018) were also retrieved from NCBI. The native chickens from these countries were separated into populations in the country level except

for Chinese native chickens that were separated in the province level. The ancient Chinese populations by Xiang *et al.* (2014) were separated into «the Central Plain» (Zhongyuan) before 40,00 years BP and «the Middle Basin Yangtze River» of Zhou dynasty (3,000-2,300 BP). The retrieved sequences were automatically aligned by the MAFFT together with the aforementioned alignment of JNC. The sites, in which more than 10% of individuals contains gaps or ambiguous bases were excluded by the in-house perl program offered by Dr. Jiaqi Wu (Tokyo Institute of Technology). Final alignment consists of 5,712 individuals with the length of 495 bp.

The nucleotide diversity within populations and the net genetic distance between populations were estimated with the p-distances using MEGA 7. The standard errors of the nucleotide diversities were estimated by the bootstrapping with 100 replications. The Neighbor Networks were estimated by the procedures as mentioned above. The East Asian and continental Southeast Asian native chickens were used for the network analyses and the South Asian and Pacific native chickens were used for the reference data of the geographic distributions of the haplotypes shared by the native chickens in Japan and other countries.

Fst matrices based on 20 nuclear microsatellite loci by Osman *et al.* (2006) and based on 30 nuclear microsatellite loci by Oka *et al.* (2010, 2011) were also used for the estimation of the Neighbor Network for the comparison of the histories of the maternal and biparental lineages.

Results

Phylogenetic relationships among JNC breeds

The neighbor network of JNC breeds based on the net distances as inferred from the complete D-loop is shown in the Fig. 1. 30 JNC breeds were clustered into five groups. The first JNC group consists of three Jidori breeds (Gifu-Jidori, Iwate-Jidori, Tsushima-Jidori) and one hybrid breed (Hinai-dori: this breed is thought to be hybrid of Jidori and Shamo, see Oana, 1951; Sato, 2011). The second JNC group consists of three Jidori breeds (Ise-Jidori, Ryujin-Jidori, Sadohige-Jidori), one Shokoku-related breed (Kurokashiwa), one Chabo- related breed (Daruma-Chabo), and one hybrid breed (Jisuri: the modern population of this breed was established by hybridizing Daruma-Chabo and Shamo, Matsuzaki, 2002). The third JNC group consists of two Jidori breeds (Tosa-Jidori, Uzura-Chabo). The fourth JNC group consists of two Jidori breeds (Aizu-Jidori, Jitokko), two Shokoku-related breed

(Onagadori, Kurekodori), one Shamo-related breed (Ko-Shamo), two hybrid breeds (Kawachi-Yakko: this breed is thought to be hybrid of Jidori and Shamo, and Satsuma-dori: this breed is thought to be hybrid of Shokoku and Shamo, see Oana, 1951; Sato, 2011), and Ukokkei. The fifth JNC group consists of one Jidori breed (Tokuji-Jidori), two Shokoku-related breeds (Shokoku, Totenko), one Shamo-related breed (Shamo), two Chabo-related breeds (Chabo, Taikan-Katsura-Chabo), three hybrid breeds (Minohiki: this breed is thought to be hybrid of Shokoku and Shamo, Minohiki-Chabo: this breed is thought to be hybrid of Jidori and Shamo, Koeyoshi: this breed is thought to be hybrid of Shamo and extinct Dai-Tomaru, see Oana, 1951; Sato, 2011), and one Dai-Tomaru-related breed (Tomaru). The neighbor network of JNC breeds based on *Fst* as inferred from D-loop also shows the essentially consistent evolutionary relationships, except for Shokoku, Totenko, and Tomaru were nested within the first group (data not shown). These five groups are not consistent with Oana (1951)'s classification, and «Jidori-related breeds», «Shokoku-related breeds» and «Shamo-related breeds» are intermingled in each other within five groups.

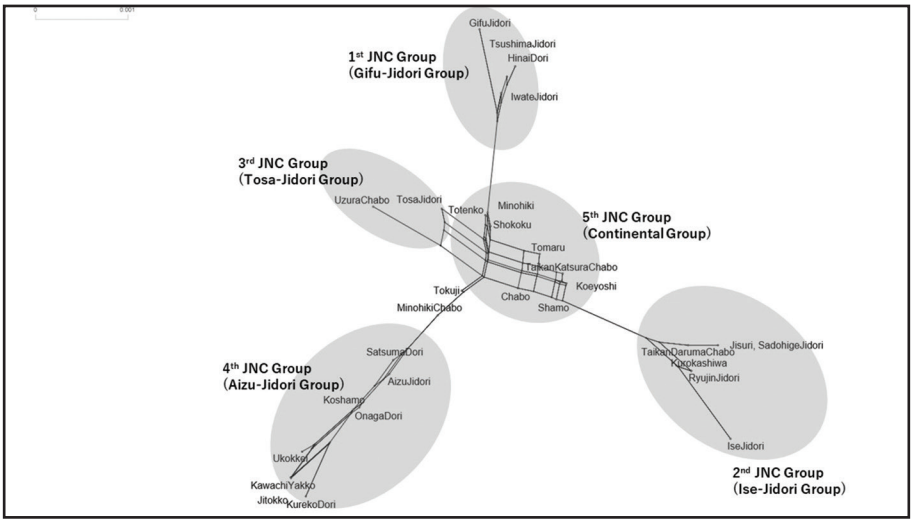

Fig. 1. *Neighbor network among the native Japanese chicken breeds based on the net genetic distances as inferred from the complete mitochondrial D-loop sequence. The lengths of the edges are proportional to the net genetic distances (substitutions per site) as shown in the scale bar.*

Phylogenetic positions of JNC breeds within East and Southeast Asian native chickens

The phylogenetic positions of JNC breeds were examined within the modern native chickens from East and Southeast Asian countries. At first, all JNC breeds were treated as one population (Japanese population), and its phylogenetic position was estimated. The neighbor network based on the net distance matrix as inferred from the partial D-loop sequences is shown in Fig. 2. The neighbor network showed a gradual geographical cline, from Southeast Asian countries to North China. This network was arbitrarily divided into three groups in accordance with geographical configurations, namely «Southeast Group», «Northeast Group», and «Central Plain Group».

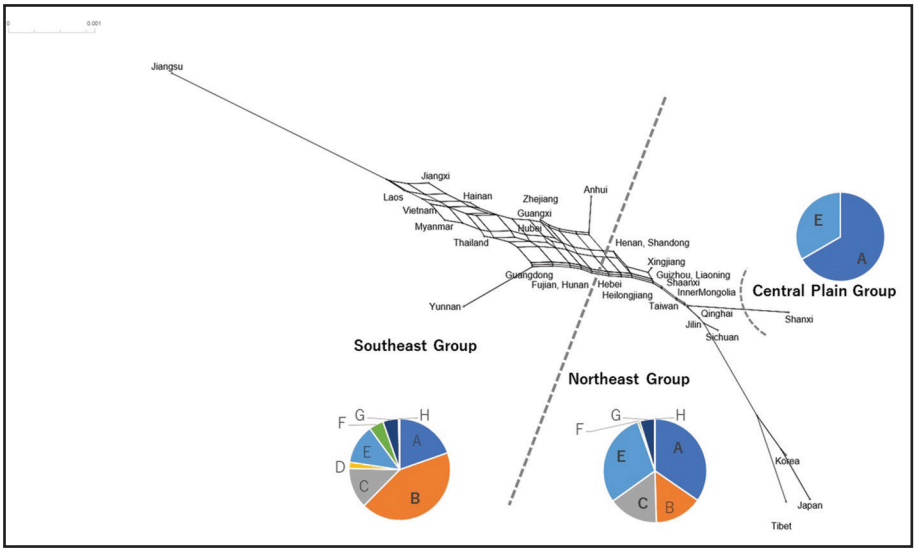

Fig. 2. *Neighbor network among the East Asian and the Southeast Asian native chicken populations based on the net genetic distances as inferred from the partial mitochondrial D-loop sequence. The lengths of the edges are proportional to the net genetic distances (substitutions per site) as shown in the scale bar. Network was separated into three groups in accordance with the phylogenetic structure as well as the geographic configurations. The pie charts indicate the compositions of the mitochondrial haplogroups in each group.*

Southeast Group consisted of the native chicken populations from Thailand, Myanmar, Laos, Vietnam, Yunnan, Guangxi, Guangdong, Hainan, Jiangxi, Fujian, Hunan, Hubei, Zhejiang, Jiangsu, and Anhui. Central Plain Group consists of Shanxi. As discussed later, the «ancient» Central Plain population was also included in this group. Northeast Group consists of Hebei (including Beijing), Henan,

Shandong, Liaoning, Jilin, Heilongjiang, Shaanxi, Inner Mongolia, Xinjiang, Sichuan (including Chongqing), Guizhou, Qinghai, Tibet, Taiwan, Korea, and Japan. Japanese population is especially close to Korean population.

The nucleotide diversity of Southeast Group is 0.0153, that of Northeast Group is 0.0152, and that of Central Plain Group is 0.0091. There are significantly different compositions of the mitochondrial haplogroups among three Groups (Fig. 2: P-value=1.3E-174). The haplogroup B is the most dominant (42.8%) in the Southeast Group, and the haplogroup A (19.6%), the haplogroup C (12.9%), the haplogroup E (12.6%), and haplogroup D (2.0%) follow it. On the other hand, the haplogroup A is the most dominant (33.0%) in the Northeast Group, and the haplogroup E (28.1%), the haplogroup C (14.8%), the haplogroup B (14.3%), and the haplogroup D (4.7%) follow it.

Subsequently, the phylogenetic positions of the each JNC breed as well as the ancient Chinese populations (Xiang *et al*., 2014) within East Asian and Southeast Asian populations were examined (Fig. 3).

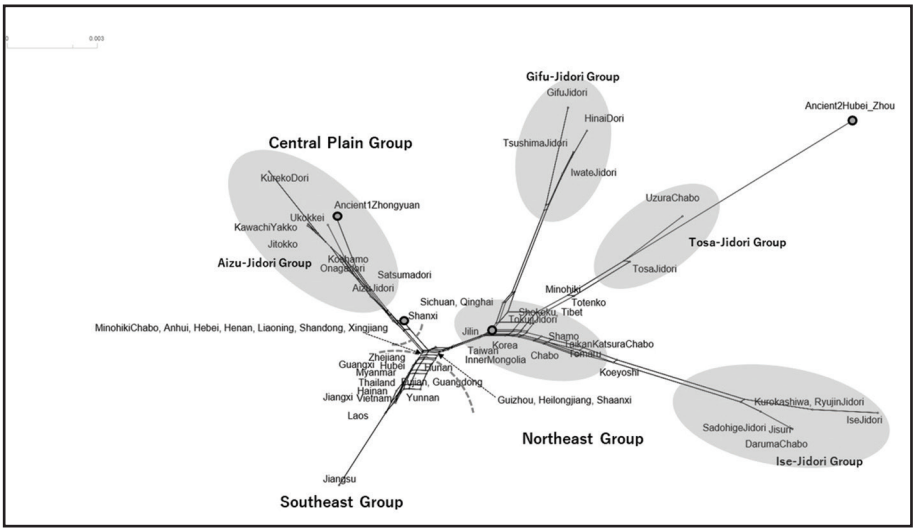

Fig. 3. *The Neighbor network depicts the phylogenetic positions of the JNC breeds among the East Asian and the Southeast Asian native chicken populations based on the net genetic distances as inferred from the partial mitochondrial D-loop sequences. The lengths of the edges are proportional to the net genetic distances (substitutions per site) as shown in the scale bar. The classification of the JNC breeds as well as the East Asian and the Southeast Asian native chickens were followed to the Figure 1 and Figure 2. The possible key populations (Korea, Shanxi, Ancient1Zhongyuan, and Ancient2Hubei_Zhou) on geographic origins of the JNC breeds (see main text) were indicated by the small gray colored circles.*

The first JNC group including Gifu-Jidori, the second JNC group including Ise-Jidori, the third JNC group including Tosa-Jidori, and the fifth JNC group including Shokoku were nested within the Northeast group, whereas the forth JNC group including Aizu-Jidori was included in the Central Plain Group together with the ancient population of Central Plain before 4,000 years BP.

Discussion

Evolutionary relationships among the JNC breeds

In this study based on the mitochondrial D-loop sequences, the JNC breeds were separated into five groups (The first to fifth JNC group in Fig. 1). These five groups based on genetic data do not reflect the three lineages (Jidori-related breeds, Shokoku-related breeds, Shamo-related breeds) defined by the morphological and archival data. Jidori-related breeds, Shokoku-related breeds, and Shamo-related breeds were highly intermingled with each other within respective group defined by the D-loop sequences except for the third JNC group consist of Jidori-lineages only (Tosa-Jidori and Uzura-Chabo). This finding largely contradicts the Oana (1951)'s classification system. On the other hand, the phylogenetic relationships based on the nuclear data show a different tendency compared to those of the mitochondrial data.

The Neighbor Networks based on the *Fst* values of nuclear microsatellite data by Osman *et al.* (2006) supports that each of the Shokoku-related breeds (Shokoku, Totenko, Onagadori, Ohiki) and the Shamo-related breeds (Shamo, Ko-Shamo, Yakido, Kinpa) forms cluster, respectively (Fig. 4). The Neighbor Networks based on Oka *et al.* (2010, 2011)'s data also show the harmonious results (data not shown).

In addition, Koeyoshi, which is thought to be the hybrid of Shamo and Dai-Tomaru, is phylogenetically close to the Shamo-related breed cluster. These results partially support Oana (1951)'s classification system. Contrarily, Jidori breeds such as Gifu-Jidori, Ise-Jidori, Tosa-Jidori, Aizu-Jidori, as well as Ehime-Jidori independently diverged, and they do not form a cluster. This finding suggests the «Jidori» defined by Oana (1951) based on the red junglefowl-like morphologically ancestral chickens are phylogenetically insubstantial group. The morphologically ancestral taxa are often misclassified as «monophyletic groups» based on the symplesiomorphies (Yonezawa *et al.*, 2017). It is plausible that «Jidori» is paraphyletic group that was introduced to and propagated in Japan in multiple times.

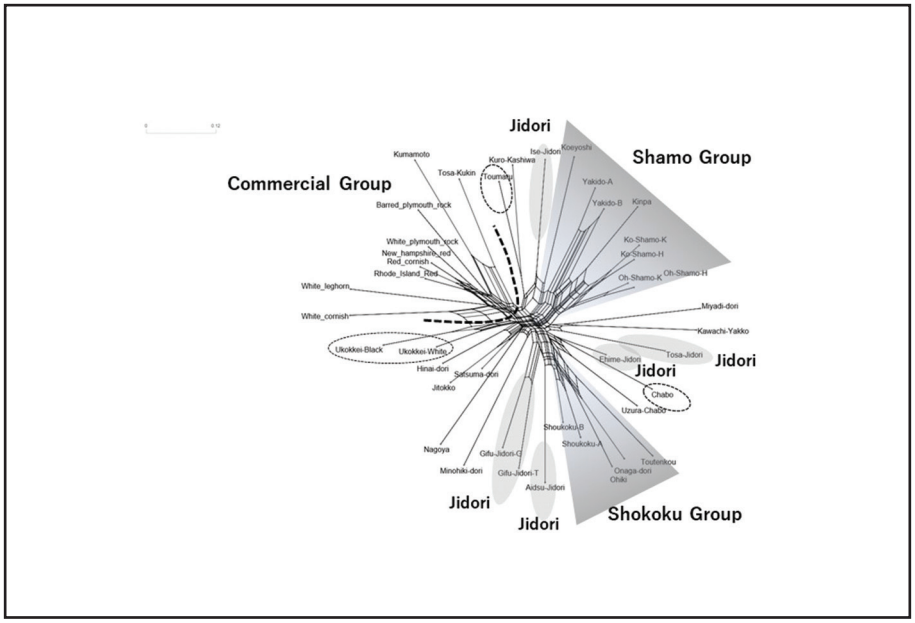

Fig. 4. *Neighbor network among the native Japanese chicken breeds based on Osman et al. (2006)'s the Fst values as inferred from the 20 microsatellite loci. The lengths of the edges are proportional to the net genetic distances (substitutions per site) as shown in the scale bar. Shokoku Group (Shokoku, Totenko, Onagadori, and Ohiki) and Shamo Groups ((Oh-)Shamo, Ko-Shamo, Kimpa, Yakido, and Koeyoshi) forms phylogenetic distinctive clusters within this network, respectively. On the other hand, Jidori Groups (Gifu-Jidori, Ise-Jidori, Tosa-Jidori, Aizu-Jidori, Ehime-Jidori), indicated by the pale gray colored ovals, does not form a phylogenetic distinctive cluster, but separated into at least four clusters and scattered within this network. The breeds (Chabo, Toumaru, and Ukokkei) thought to be introduced in Edo Era were indicated by the dashed lined ovals.*

Interestingly, if we focus on the Jidori breeds, the phylogenetic relationships based on mtDNA (Fig. 1) and based on the nuclear microsatellite loci (Fig. 4) show similar branching patterns. The first JNC group includes Gifu-Jidori, the second JNC group includes Ise-Jidori, the third JNC group includes Tosa-Jidori, and the fourth JNC group includes Aizu-Jidori. Therefore, hereafter, we would like to refer these four JNC groups as Gifu-Jidori group, Ise-Jidori group, Tosa-Jidori group, and Aizu-Jidori group, respectively. The fifth JNC group mainly consists of the breeds propagated to Japan from continent in Heian Era to Edo Era such as Shokoku (and its related breeds), Shamo, Chabo, and Tomaru. Therefore, hereafter we would like to refer the fifth JNC group as the Continental group.

There remains an outstanding question. Why do the Gifu-Jidori group, the Ise-Jidori group, and the Aizu-Jidori group contain Shokoku-related breeds such as Onagadori, Kurekodori, Shamo-related group

such as Koshamo, and other breeds such as Chabo-related breed (Daruma-Chabo) and Ukokkei (Fig. 1)? The plausible explanation of the discrepancy between the nuclear and mitochondrial genes is that when these breeds were established in Japan, several Jidori breeds, especially female Jidori were largely involved in the crossbreeding, with repeated backcross mainly by the males of Shokoku or Shamo.

Phylogeographic structure of East and Southeast Asian native chickens

This study demonstrated a geographical cline on the genetic compositions of the native chickens in East and Southeast Asian countries from the south to north. Previous works (Akishinonomiya *et al*., 1996; Wang *et al*., 2020) suggested the Southeast Asia and South China was one of the domestication centers of the chickens. Does this geographical cline represent the northward dispersal process of the domestic chicken from the Southeast Asia region including South China? It is accepted that genetic diversity of domestic animals generally declines along the geographic distances from the point of domestication origin (Troy *et al*., 2001; Beja-Pereira *et al*., 2004). However, different genetic compositions and diversities seen in Southeast Group and Northeast Group does not support this scenario. Indeed, the nucleotide diversities of the Southeast Group (0.0153) and Northeast Group (0.0152) are essentially same level. If we focused on the nucleotide diversities in each haplogroup, those of the haplogroup B (the most major haplogroup in Southeast Group) and the haplogroup D are higher in the Southeast Group but haplogroup A, haplogroup C, and haplogroup E are higher in the Northeast Group (Fig. 5). These latter three haplogroups are the dominant haplogroups in the Northeast Group as mentioned above. In addition, the frequencies of the haplogroup A and haplogroup C are very low in the red junglefowl populations (Miao *et al*., 2013). If the domestic chickens merely originated in the Southeast Asian region including South China and disperse to North China, this tendency cannot be explained. Instead, this tendency would support that the Northeast Group originated from distinct unknown wild populations as suggested by previous studies (Xiang *et al*., 2014; Huang *et al*., 2018, but see also Eda *et al*., 2016; Peters *et al*., 2016).

The Northeast Group is fundamentally distributed on the northern side of the Yangtze river. However, they are also found in the lower latitude of the highlands (Sichuan, Guizhou, Qinghai, and Tibet) and islands (Taiwan and Japan). This finding suggests the Northeast Group was widely distributed in the North China and its surrounding regions in the passed time. Subsequently, the Southeast Group spread

northward along the lowland of the East China. These two Groups merged through gene introgressions and formed the geographic clines observed in genetic level. Probably, a nearly pure genetic component of the Northeast Group was retained in the geographically more remote areas such as Tibet, Korea, and Japan.

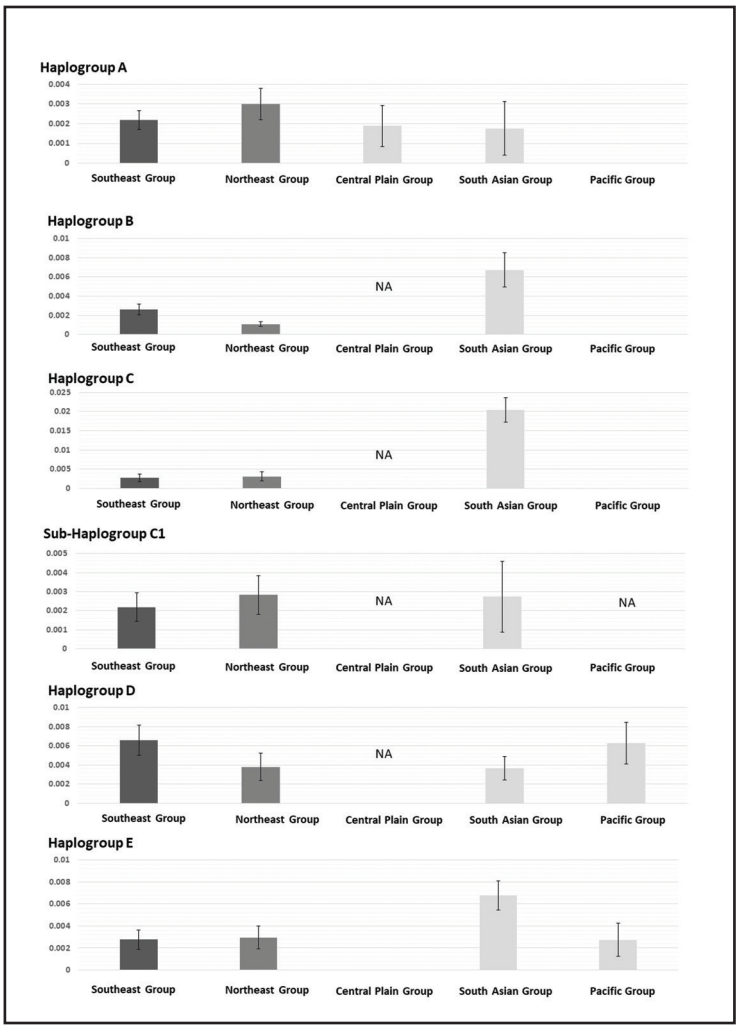

Fig. 5. *Nucleotide diversities within each haplogroup observed in the native chickens in the East and the Southeast Asian countries. Geographic Groups (Southeast Group, Northeast Group, Central Plain Group) were followed to Figure 2 and 3. In addition, South Asian (India and Sri Lanka) and Pacific (Indonesia, Guam, and Vanuatu) populations were added in this analysis. The error bars indicate the standard errors estimated by the bootstrapping (100 replications). NA (not applicable) indicates the absences of the haplogroup in given geographic group.*

The geographic origins and histories of JNC breeds

Based on the findings in this study and insights from previous works (Oana, 1951; Komiyama *et al*., 2003, 2004; Osman *et al*., 2006; Oka *et al*., 2007, 2010, 2011; Eda, 2018), we suggest a novel hypothesis for the origin and evolution of the JNC breeds as follows:

Phase 1: Origins of Jidori breeds

There are no reliable archaeological remains of domestic chicken in Japan before the Jomon Era (14,000-1,000 BC). The oldest remains are reported from the Middle to the Late Yayoi Era, and therefore the propagation of chickens to Japan is thought to be from the Yayoi Era (Eda, 2018). The sex ratios of chicken remain in the Yayoi Era are highly biased toward males (Eda, 2018). Therefore, it might have been difficult to sustain Japanese chicken populations for many generations and it is likely that that domestic chickens were repetitively introduced into Japan from surrounding countries such as Korea and China (Eda, 2018).

Molecular evidence (Osman *et al*., 2006, this study) suggests that Jidori breeds consist of the four distinct lineages, namely Gifu-Jidori Group, Ise-Jidori Group, Tosa-Jidori Group, and Aizu-Jidori Group. The genetic component of Aizu-Jidori Group is close to those of the Central Plain Group (Shanxi Province) as well as the ancient population of Central Plain (Shandong and Hebei) before 4,000 years BP. On the other hand, Gifu-Jidori Group, Ise-Jidori Group, and Tosa-Jidori Group derived from the Northeast Group (Fig. 3). This finding implies the Jidori breeds were introduced into Japan in multiple times.

Regarding Aizu-Jidori Group, the Hap3 (haplogroup A), the dominant haplotype seen in Aizu-Jidori and Jitokko, is widely distributed in Shanxi Province and its surrounding region at high frequencies (Tab. 1). It is possible the ancestor of the Aizu-Jidori Group originated in the ancient Chinese Central Plain (Hebei, Henan, Shanxi, and Shandong). The exact timing and route of their introduction into Japan are unknown. However, considering the wide geographic distribution of the breeds in Aizu-Jidori Group, as well as the several important JNC breeds such as Onagadori, Kurekodori, Ko-Shamo are included in this Group, it is plausible the ancestral population of this group was widely introduced into Japan from Kyushu, Shikoku to the northeast of Honshu, and several Jidori breeds represented by Aizu-Jidori and Jitokko were established in each region. Then, they were involved in the crossbreeding with Shokoku or Shamo for establishing derived breeds such as Onagadori, Kurekodori, and Ko-Shamo.

			A			B	C		D			E					
			hap1	hap2	hap3	hap4	hap5	hap6	hap7	hap8	hap9	hap10	hap11	hap12	hap13	hap14	hap15
Native Japanese chicken breeds	Jidori	Tosa-Jidori	0	0	0	0	0	0	**0.8**	0	0.05	0.15	0	0	0	0	0
		UzuraChabo	0	0	0	0	0	0	**0.333333**	0	0	0	0	0	0	0	0
		Ise-Jidori	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0
		Gifu-Jidori	0	0	0	0	0	0	0	0	0	0	0	0	**1**	0	0
		Iwate-Jidori	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0
		Tsushima-Jidori	0	0	0	0	0	0	0	0	0	**1**	0	0	0	0	0
		Ryujin-Jidori	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0
		Sadohige-Jidori	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0
		Aizu-Jidori	0	0	**0.733333**	0	0	0.066667	0	0	0	0	0	0.2	0	0	0
		Tokuji-Jidori	0.166667	0	0.166667	0	0	0	0	0	**0.666667**	0	0	0	0	0	0
		Jitokko	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0
	Shokoku	Shokoku	0.25	0	0.125	0	0	0	0	0	0	**0.625**	0	0	0	0	0
		Totenko	0.2	0	0	0	0	0.1	0	0	0	**0.4**	0	0	0.3	0	0
		Onagadori	**0.571429**	0	0.285714	0	0	0	0	0	0	0.142857	0	0	0	0	0
		Kurekodori	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Kurokashiwa	0	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0
	Shamo	Shamo	0	0	0.033333	0.2	0	**0.533333**	0	0	0	0.133333	0	0	0	0	0
		Koshamo	**0.5**	0	0.375	0	0.125	0	0	0	0	0	0	0	0	0	0
	Chabo	Chabo	0	0	**0.142857**	0	**0.142857**	**0.142857**	**0.142857**	**0.142857**	0	**0.142857**	0	0	0	0	0
		TaikanKatsuraChabo	0	0	0	0	0	**0.5**	0	0	0	0	0	0	0	0.166667	0.166667
		DarumaChabo	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
	Tomaru	Tomaru	0	0	0	0	0	0.444444	0	0	0	**0.555556**	0	0	0	0	0
	Ukokkei	Ukokkei	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0	0
	others	Koeyoshi	0	0	0	0	0	**0.6**	0	0	0	0	0	0.4	0	0	0
		Satsumadori	0	0	**0.7**	0	0	0.3	0	0	0	0	0	0	0	0	0
		Jisuri	0	0	0	0	**1**	0	0	0	0	0	0	0	0	0	0
		Hinaidori	0	0	0	0	0	0	0	0	0	**0.5**	0.333333	0	0	0	0
		Minohiki	0	0	0	0	0	0	**0.6**	0	0	0	0	0	0.4	0	0
		KawachiYakko	0	0	**1**	0	0	0	0	0	0	0	0	0	0	0	0
		MinohikiChabo	**0.5**	0	0	0	0	0	**0.5**	0	0	0	0	0	0	0	0
Native Asian chickens	Northeast	Korea	0	0	0.125	0.083333	0.041667	0.138889	0	0	0.013889	0.416667	0.013889	0	0.013889	0	0
		Jilin	0	0	0.1875	0.1875	0	0.25	0	0	0	0.3125	0	0	0	0	0
		Heilongjiang	0	0	0.333333	0.266667	0	0.066667	0	0	0	0.2	0.066667	0	0	0	0
		Liaoning	0	0	0.315789	0.157895	0	0.263158	0	0	0	0.263158	0	0	0	0	0
		Taiwan	0	0	0.375	0.125	0	0.125	0	0	0	0.25	0.125	0	0	0	0
		Xingjiang	0	0.015873	0.365079	0.142857	0.111111	0.111111	0.015873	0	0.015873	0.095238	0	0	0	0	0
		InnerMongolia	0	0	0.466667	0.1	0	0.166667	0	0	0	0.133333	0	0	0.1	0	0
		Shaanxi	0	0	0.26087	0.304348	0	0	0	0	0	0.391304	0.043478	0	0	0	0
		Tibet	0	0	0.202479	0.061983	0.008264	0.028926	0	0	0.012397	0.268595	0.012397	0	0	0	0
		Qinghai	0	0	0.285714	0.142857	0	0	0	0	0	0.285714	0.071429	0.071429	0.071429	0	0
		Sichuan	0	0.002053	0.36345	0.084189	0.00616	0.022587	0	0	0	0.273101	0.061602	0	0.010267	0	0
		Guizhou	0	0.014085	0.338028	0.183099	0	0.042254	0	0	0	0.183099	0.014085	0	0	0	0
		Hebei	0	0.030769	0.169231	0.307692	0.092308	0.030769	0	0	0	0.138462	0.015385	0	0	0	0
		Henan	0	0.077151	0.249258	0.219585	0.041543	0.109792	0	0	0	0.074184	0.014837	0.005935	0	0	0
		Shandong	0	0	0.198312	0.248945	0.063291	0.080169	0.008439	0	0	0.101266	0.016878	0.004219	0	0	0
		Ancient2Hubei_Zhou	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Central Plain	Ancient1Zhongyuan	0	0.333333	0	0	0	0	0	0	0	0	0	0	0	0	0
		Shanxi	0	0	0.416667	0	0	0	0	0	0	0.333333	0	0	0	0	0
	Southeast	Anhui	0	0	0.205357	0.232143	0.125	0.178571	0	0	0	0.008929	0	0	0	0	0
		Fujian	0	0	0.116883	0.329004	0	0.082251	0.038961	0	0	0.298701	0.012987	0	0	0	0
		Guangdong	0	0	0.055914	0.402151	0.036559	0.122581	0	0	0	0.210753	0.004301	0.002151	0	0.004301	0
		Guangxi	0.018433	0	0.179724	0.400922	0.009217	0.110599	0	0	0	0.152074	0	0	0	0	0
		Hainan	0	0	0.1	0.525	0	0.125	0	0	0	0.15	0	0	0	0	0
		Hubei	0	0.013793	0.241379	0.365517	0.027586	0.137931	0	0	0	0.068966	0.006897	0	0	0	0
		Hunan	0.003378	0.047297	0.108108	0.314189	0.054054	0.135135	0	0	0	0.179054	0.006757	0	0	0	0
		Jiangsu	0	0.076923	0.046154	0.738462	0	0.092308	0	0	0	0.030769	0	0	0	0	0
		Jiangxi	0.003914	0.015656	0.227006	0.440313	0.02544	0.062622	0	0	0	0.062622	0.001957	0	0.021526	0	0.001957
		Zhejiang	0	0.004878	0.146341	0.419512	0.009756	0.219512	0	0	0	0.097561	0	0	0	0	0
		Yunnan	0	0.002597	0.193506	0.168831	0	0.014286	0	0	0	0.049351	0.007792	0	0.001299	0	0
		Laos	0	0	0.217391	0.391304	0	0	0.014493	0	0.014493	0	0.028986	0	0	0	0
		Myanmar	0	0	0.315789	0	0	0	0	0	0	0.052632	0	0	0	0	0
		Thailand	0	0	0.208333	0.208333	0	0	0.166667	0	0	0	0	0	0	0	0
		Vietnam	0	0	0.172764	0.355691	0	0.034553	0.042683	0	0.00813	0.052846	0.006098	0	0.002033	0	0
	South Asia	India	0	0.00885	0.011799	0.011799	0	0.0059	0.014749	0	0.017699	0.454277	0.00295	0.179941	0.00295	0	0
		SriLanka	0	0	0.047619	0.071429	0.02381	0	0	0	0.02381	0.309524	0.02381	0.071429	0	0	0
	Pacific	Guam	0	0	0	0	0	0	0.2	0	0	0.4	0	0	0	0	0
		Vanuatu	0	0	0.02439	0	0	0	0	0.146341	0	0.02439	0	0	0	0	0
		Indonesia	0	0	0	0.230769	0	0	0.076923	0	0.076923	0.384615	0	0	0	0	0

Tab. 1. *Frequencies of haplotype shared by JNC and Asian countries.The frequencies of the dominant haplotypes in each JNC breed were indicated by the bold fonts. The frequencies of the haplotypes in each Asian native chicken populations (except for JNC breeds) were indicated by the color scales.*

Our phylogenetic network indicates that Gifu-Jidori Group, Ise-Jidori Group, and Tosa-Jidori Group were derived from the Northeast Group (Fig. 3). They are especially close to Jilin (Northeast China), Korea, and Tibet. Based on the geographical configurations and archaeological record (Eda, 2018), it is plausible these Jidori groups (except for Tosa-Jidori Group as discussed later) were propagated to Japan from Korea. To evaluate this hypothesis, the geographical distributions of the haplotypes dominant in these Jidori groups were examined (Tab. 1). As

for Gifu-Jidori Group, despite the Hap10 (haplogroup E), the dominant haplotype of Iwate-Jidori and Tsushima-Jidori, is widely distributed in Asia including India and Indonesia, this haplotype is also distributed in Korea with high frequency (41.7%: Table 1). Hap13 (haplogroup E), is the dominant haplotype of Gifu-Jidori. It is rare but a widely distributed haplotype in China and India. Although its haplotype frequency is low in South China and India (<1%, except for Jiangxi at 2.2%). This haplotype has a frequency of 1~10% in Northeast Group including Korea (1.4%). As for the Ise-Jidori Group, the Hap6 (haplogroup C), is the dominant haplotype in Ise-Jidori and Ryujin-Jidori and is widely distributed in China and India. The haplotype frequencies are relatively high (>25%) in Northeast China (Liaoning and Jilin), and in Korea (13.9%). The Hap5 (haplogroup C), the dominant haplotype in Sadohige-Jidori, is also found in Korea (4.2%).

Ones of the oldest archaeological records of the Japanese chicken remains are from Iki island (Karakami Shell Mounds and Tsujinohara Ruin: Middle to Late Yayoi Era: Eda, 2018) of the Tsushima Strait. This island is nearby Tsushima island where Tsushima-Jidori (Gifu-Jidori Group) originated. On the other hand, chicken remains in Korean Peninsula can be traced back to Neolithic. It is plausible that the Gifu-Jidori Group and the Ise-Jidori Group were introduced from Korea repetitively after Yayoi Era. Gifu-Jidori Group widely expanded within Japan from Tsushima island (Tsushima-Jidori) to Northeast of Honshu (Iwate-Jidori). On the other hand, the Ise-Jidori Group is mainly distributed in the Central Honshu (Ise-Jidori, Ryujin-Jidori, and Sadohige-Jidori). However, non-Jidori breeds included in Ise-Jidori Group can be also found in West Honshu (Kurokashiwa) and Kyushu (Daruma-Chabo and Jisuri).

The origin of Tosa-Jidori Group is enigmatic. The dominant haplotype (Hap7, haplogroup D) of Tosa-Jidori Group (Tosa-Jidori and Uzura-Chabo) is not found in Korea and Northeast China (Jilin, Liaoning, Heilongjiang). The haplogroup D itself is found in very low frequency in North and South China (Miao *et al.*, 2013). It is possible the phylogenetic position of Tosa-Jidori Group within the Northeast Group is analytical artifact caused by the genealogical closeness of the haplogroup C (abundant in the North Chinese Group) and the haplogroup D. Oka *et al.* (2007) suggested the possibility that Tosa-Jidori Group was propagated from Southeast Asia. Miao *et al.* (2013) demonstrated the haplogroup D is especially abundant in Pacific group including Indonesia (see also Tab. 1). On the other hand, the haplogroup D tentatively expanded in the Middle Basin Yangtze River of Zhou

dynasty (Xiang *et al.*, 2014), and Hap7 can be also detected in China (Xinjiang, Shandong, and Fujian) with low frequencies (0.8~3.9%: Tab. 1). Since the resolution of haplotype classification based on the partial mitochondrial D-loop is too low to elucidate the genetic structure of this haplogroup, the detailed phylogeographic analysis based on the mitochondrial genome is prerequisite to clarify the enigmatic origin of Tosa-Jidori Group. Such research is now in progress.

Phase 2: Origin of Shokoku-related breeds

Shokoku is an aesthetic breed generally believed to be imported from China. Their beautiful and characteristic form can be seen in Chōjū-Giga (Animal-person Caricatures) from the 12th to 13th centuries (The Late Heian Era to the Early Kamakura Era) (Oana, 1951). It indicates the introduction time of Shokoku is by Heian Era. Although there are several hypotheses on their detailed geographical origin, it is generally believed that they were introduced to Japan from Zhoushan, formerly called «Changguo (昌國)» in Zhejiang. The breed name «Shokoku (小國)» is same pronunciation with «Changguo» in Japanese language (Sato, 2011).

Our molecular data suggest Shokoku and its related breed (Totenko) belong to the Northeast Group but this result is inconsistent with historical records. In addition, the dominant haplotype of Shokoku and Totenko is the Hap10 (haplogroup E), which is also dominant in Iwate-Jidori and Tsushima-Jidori. This haplotype is widely distributed in Asia including India and Indonesia as discussed above. Then, we calculate the likelihood that the haplotypes observed in Shokoku and Totenko (Hap1, Hap3, Hap 6, and Hap 10) geographically originated based on the haplotype frequency in each region under the assumption that (1) Shokoku and Totenko originated in same locality and (2) all populations have retained the current haplotype frequencies from the introduction time of Shokoku and Totenko. Jiangxi was selected as the best candidate of Shokoku's geographical origin based on our maximum likelihood approach. Since Zhoushan (Changguo) and neighboring Ningbo are historically famous trading ports in China, it is possible that the ancestor of Shokoku originated in the area geographically bordering with Zhejiang such as Jiangxi. After their introduction to Japan, Shokoku widely spread to many regions in Japan, and several important breeds such as Onagadori, Kurekodori, Kurokashiwa were established by crossbreeding with the Jidori breeds in each locality.

Phase 3: Origin of Shamo-related breeds

Shamo is generally believed to be from Thailand during the Edo Era. The breed name «Shamo» is thought to be from «Siam», the former name of Thailand. However, our phylogenetic network suggests the genetic component of Shamo is similar to the Northeast Group, rather than Southeast Asian Group (Fig. 3). The Hap 6, the dominant haplotype in Shamo (Tab. 1) belongs to the sub-haplogroup C1 that is more common and probably originated from the North China (Huang *et al*., 2018).

Previous studies (Komiyama *et al*., 2003, 2004, Oka *et al*., 2007) suggested that Shamo shares haplotypes with Jidori breeds or Shokoku-related breeds (Tab. 1). These molecular evidences imply the ancestor of Shamo was introduced into Japan in earlier times. Komiyama *et al.* (2003) pointed out that chickens with the characteristic morphological features such as pea comb, long neck and upright posture are seen in drawings for were Choju-Giga (Late Heian Era to Early Kamakura Era). In addition, the drawings of Shamo-like chicken can be also seen in the Nenju-Goji-Emaki established in the Late Heian Era.

It is notable that the very rare haplogroup H which is only distributed in Yunnan and Thailand is also found in Shamo from Okinawa (Southwest Japan) (Oka *et al*., 2007; Miao *et al*., 2013; Teinlek *et al*., 2018). In addition, the second dominant haplotype in Shamo (Hap4) belongs to haplogroup B, which is more abundant in Southeast Group rather than the Northeast Group. This haplotype can be observed only in Shamo in JNC breeds (Tab. 1). These results suggest there was genetic introgression to Shamo from the chicken from the Southeast Group. It is possible the ancestral fighting cocks were introduced into Japan from the Northeast Group by the Heian Era. Then this fighting cock was improved in a multi-layered way by the crossbreeding with chickens from Southeast Asia such as Thailand introduced in Edo era. Several breeds such as Ko-shamo might have been established by the crossbreeding with Jidori breeds.

Phase 4: Origin of Chabo-related breeds, Tomaru, Ukokkei

Chabo, Dai-Tomaru, Ukokkei are also generally thought to be introduced to Japan in Edo Era through trading with East Asia and Southeast Asia (Sato, 2011). The breed name of Chabo is generally thought to be from «Champa», the kingdom located in central and southern Vietnam from the 2nd century to 1,832. However, as seen in Shamo, the genetic component of Chabo is similar to the Northeast Group, rather than the Southeast Group (Fig. 3). It is possible that the ancestral population

of Chabo was also introduced to Japan from the Northeast Group such as China or Korea. Notably, Chabo have the haplotype (Hap 8, the haplogroup D) only shared with the Pacific population, but not reported from China (Tab. 1). This finding suggests Chabo was also established in a multi-layered breeding way by crossing with chickens from Pacific populations.

Dai-Tomaru is an extinct breed believed to be introduced from China in the Edo Era. Since they already went extinct, it is difficult to present a realistic picture of this breed. It is even possible «Dai-Tomaru» is not a breed, but generic name of breeds of large body sized chickens with black feathers imported from China. The modern breed Tomaru is thought to have been established by the crossbreeding of Dai-Tomaru and the local long crowing chickens (Oana, 1951; Sato 2011). In our study, the genetic component of Tomaru is also similar to those of the Northeast Group. The dominant haplotype of Tomaru (Hap 10) can be seen in Shokoku, Totenko, Iwate-Jidori, Tsushima-Jidori, and Hinaidori in high frequency (Tab. 1). The second dominant haplotype of Tomaru (Hap 6) can be seen in Shamo, Taikan-Katsura-Chabo, Kurokashiwa, Ise-Jidori, Ryujin-Jidori, and Koeyoshi in high frequency. Therefore, interestingly, Tomaru shares the haplotypes with Japanese representative long crowing chickens, Totenko and Koeyoshi.

Ukokkei is Japanese variety of the White Silkie, and this breed name is Japanese pronunciation of Wuguji (black boned chicken) in Chinese character. The haplotype seen in Ukokkei (Hap2, haplogroup A) is rare, but distributed along the Yangtze River (Tab. 1). It is possible Ukokkei originated from this area. Although our phylogenetic network suggests Ukokkei belongs to Aizu-Jidori Group, Ukokkei do not share the haplotype with other JNC breeds (Tab. 1). It is possible their phylogenetic position is an analytical artifact, and Ukokkei and Aizu-Jidori Group were independently introduced to Japan from China, and Ukokkei have been kept without crossbreeding with Aizu-Jidori Group.

Conclusions

The scenario suggested by this study based on molecular evidence is largely harmonious with the Oana (1951)'s hypothesis. However, at the same time, our results indicated more a complicated history of the origin and evolution of JNC breeds. Jidori breeds can be separated into four group (Gifu-Jidori Group, Ise-Jidori Group, Tosa-Jidori Group, and Aizu-Jidori Group), and they were probably introduced into Japan

multiple times from China and Korea. However, the geographical origin of Tosa-Jidori Group has remained an enigmatic issue. Later, Shokoku was introduced to Japan from East China, and several breeds were established directly (Totenko) or through crossbreeding with Jidori breeds (Onagadori, Kurekodori, Kurokashiwa). Although Shamo and Chabo are generally believed to have originated in Southeast Asia, their genetic components are similar to Northeast Group rather than the Southeast Group. However, Shamo and Chabo possess the haplotype characteristic in the Southeast Group or Pacific population. This result suggests their multi-layered origins. In this study, we illustrated the entire picture of the history on the origins and evolutions of JNC breeds based on the comparisons with thousands of native chickens from Asia. However, the resolution of the picture based on the analysis of mitochondrial D-loop data is still relatively low. Recently, population genomic study based on 863 chickens and red junglefowls were conducted to elucidate the domestication origin of chickens (Wang *et al*., 2020). In the future, histories of JNC breeds suggested in this study should be examined and tested using whole genomic scaled data.

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Corresponding author: Takahiro Yonezawa: ty205685@noda.ac.jp

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Rare allele sharing in the East Asian

JUN GOJOBORI*

Parole chiave: relazioni genetiche, SNP, ipotesi di struttura doppia giapponese.

Riassunto — Gli alleli rari sono varianti che vengono trovate nella popolazione a frequenze molto basse. e che dovrebbero essere più recenti degli alleli comuni con frequenze moderate. Due popolazioni con un allontanamento recente, condividono di regola più alleli di quelli condivisi da popolazioni che si sono separate da un tempo più lungo. Perciò lo studio del numero di alleli rari condivisi può chiarire le relazioni genetiche fra popolazioni. In questo studio ho utilizzato i dati sulla condivisione di alleli rari nelle popolazioni dell'Asia orientale, con particolare attenzione a quella giapponese per esaminare se l'analisi di alleli rari possa riprodurre la relazione tra queste popolazioni. Ho usato i dati di 1000 genomi, dei quali i cinesi di Beijing come popolazione di riferimento, e altre quattro popolazioni come test. Il risultato della condivisione di alleli rari riproduce la nota relazione popolazionale con eccezione della popolazione di Tokyo. Tale differenza può essere spiegata con l'ipotesi di doppia struttura di Hanihara. Riteniamo che sia possibile utilizzare lo studio degli alleli rari per studiare la storia della popolazione Giapponese in maggiore dettaglio.

Key words: genetic relationships, SNP, dual structure hypothesis of Japanese.

Summary — Rare alleles are variants found in a population at very low frequency, such as singletons. The ages of these rare alleles should be younger than the common alleles with moderate frequencies. When two populations recently diverged they should share more rare alleles compared with when populations that have older split times. Therefore, the number of shared rare alleles can elucidate the genetic relationship among populations. I applied rare allele sharing to East Asian populations, with the particular attention to the Japanese population, to examine whether rare allele analysis can reproduce the relationship between these populations. I used 1000 genome project data, and Chinese in Beijing as a reference population and four other populations as a test. The result of rare allele sharing reproduce the known population relationship except for the Japanese population from Tokyo. This difference can be explained by the Dual structure hypothesis by Hanihara. It is possible that rare allele sharing can be used to study Japanese population history in more details.

Background

Rare alleles are alleles with very low frequencies in a population. One example of rare alleles is singletons when only one chromosome carrying a mutation is found in the population. Because rare alleles

* Department of Evolutionary Studies of Biosystems, SOKENDAI (The Graduate University for Advanced Studies), Kanagawa, Japan.

or singletons have very low frequencies, the time when the mutation arose should be more recent than alleles of middle or high frequencies (Kimura *et al*., 1973; Griffiths *et al*., 1998; Slatkin *et al*., 2000). When you have multiple population samples, each population should have its own singletons and two populations can share their singletons between populations (the SNP is now globally doubleton). This is the one of the cases of rare allele sharing. When the two populations have more recently diverged then the degree of the shared rare allele is higher compared to populations that have a more remote split time. This feature of rare allele sharing means that the genetic affinity of two populations can be estimated by simply counting the number of shared rare allele between populations (Schiffels *et al*., 2016; Flegontov *et al*., 2019). This method can save computational power and time when the demographic history of a population is simulated because no information is required on middle or high frequency class mutations with older origins (Schiffels *et al*., 2016; Flegontov *et al*., 2019). It was also shown that rare allele sharing is a powerful tool when ancient samples are considered. When the ancient samples of ancestral populations who is not yet subject to admixture are available, the rare allele sharing provide a very simple method to infer the ancestry of extant populations (Schiffels *et al*., 2016; Flegontov *et al*., 2019). However, not all samples of ancient genomes have enough depth to call variants with accuracy. Although the number of SNPs found in a sample can be normalized by using outgroups, low sequence quality of ancient DNA can reduce the number of rare alleles called and further affect the result of rare allele sharing.

In this paper, I focus on rare allele sharing among East Asian populations with special interest in the Japanese population. The Jomon period in Japan started around 16,000 years ago and continued until the beginning of Yayoi period. During Jomon period, people were hunter-gatherer. They kept this lifestyle for more than 10,000 years which is somewhat unique to Jomon (Taniguchi, 2017; Lucquin *et al*., 2018). The Yayoi period started around 10th century BC when wet-field rice cultivation began in North Kyushu (Hudson, 1992; Sahara, 1992; Fujio, 2015; Stevens *et al*., 2017; Miyamoto, 2019). Rice culture was brought to the Japanese archipelago by Yayoi migrants from the mainland. Skull bone morphology in the Yayoi period appeared to change from that of the Jomon period (Hanihara, 1991). Hanihara (1991) proposed a «Dual structure hypothesis» to explain the change of morphological features in the people of Yayoi and Jomon. He also explained the establishment of the current Japanese population based on phenotypic

data. According to the Dual structure hypothesis, the current Japanese population is the descendant of people in Yayoi period when there was admixture between the indigenous Jomon population and Yayoi migrants (Hanihara, 1991). The Dual structure hypothesis also explains the variation within the Japanese population (such as West vs East) due to the varying contribution of Yayoi migrants. Hanihara also tried to explain the apparent shared traits between Ainu population in Hokkaido region and Ryuku population in Okinawa region despite the large distance between them. He proposed that because Hokkaido and Okinawa are at the edges of Japanese Archipelago, the impact of Yayoi migrants was smaller in those regions. As a result, Ainu population and Ryuku population retained higher component of Jomon ancestry and shared similar traits that were derived from Jomon. The Dual structure hypothesis is supported by genetic data (Omoto *et al.*, 1997; Japanese Archipelago Human Population Genetics Consortium *et al.*, 2012). Recently, the proportion of Jomon components was estimated using whole ancient genome sequencing of Jomon period individual. The Jomon contribution was estimated as 10%-40% (Jinam *et al.*, 2015; Nakagome *et al.*, 2015; Kanzawa-Kiriyama *et al.*, 2017).

My aim was to apply the rare allele sharing to extant East Asian populations and examine whether rare allele sharing can mirror the relationship among East Asians referred from genome wide SNPs data and whether it is an applicable test of the population admixture history such as that of the Japanese population.

Genotype data and procedure to obtain rare allele sharing

High coverage genotype data (30x) of 1000 genome project (1KG) were retrieved from https://www.internationalgenome.org/data-portal/data-collection/30x-grch38. CDX (Chinese Dai in Xishuangbanna), CHB (Han Chinese in Beijing), CHS (Southern Han Chinese), JPT (Japanese in Tokyo), KHV (Kinh in Ho Chi Minh City) and YRI (Yoruba in Ibadan) genotype data were extracted in bcf format files (The 1000 Genomes Project Consortium, 2015). CHB and YRI are reference populations and CDX, CDS, JPT and KHV are test populations for rare allele sharing analysis. In order to extract singletons, I used bcftools view function with options of «-c 1 -C 1 –snp» for each of five bcf files (Li, 2011). The number of shared rare allele with CHB or with YRI were counted for each samples of test populations CDX, CHS, JPT and KHV. The degree of rare allele sharing with CHB for each sample was calculated as (the number of singletons shared with CHB) / (the number of singletons

shared with YRI).

Results of rare allele sharing

I retrieved high coverage genotype data from 1KG. Shared rare allele with CHB and with YRI were counted for each sample in CDX, CHS, JPT and KHV, respectively. To compare the degree of rare allele sharing with CHB between test populations, I normalized the count of shared singletons with CHB by that of YRI. This procedure allows the difference of genotype SNPs among samples to be normalized. For 1KG, there might not be a large difference among samples. But when it comes to ancient samples, which is one of the applications of rare allele sharing analysis, this normalization process is needed. This is because the sequenced ancient DNA might not sufficiently cover all genomic regions and the result is that the number of SNPs is less. When there is difference of SNPs in genome data derived from low quality samples (such as ancient samples) and from high quality samples (such as extant sample), normalization of the number of SNPs is needed. It can be assumed that the genetic distance between African populations and each of East Asian population is large and does not significantly differ between East Asian samples. Therefore, I normalized the share count of shared singletons with CHB by that of YRI. Fig. 1 shows the distribution of the degree of rare allele sharing with CHB, normalized by that of YRI for four test populations.

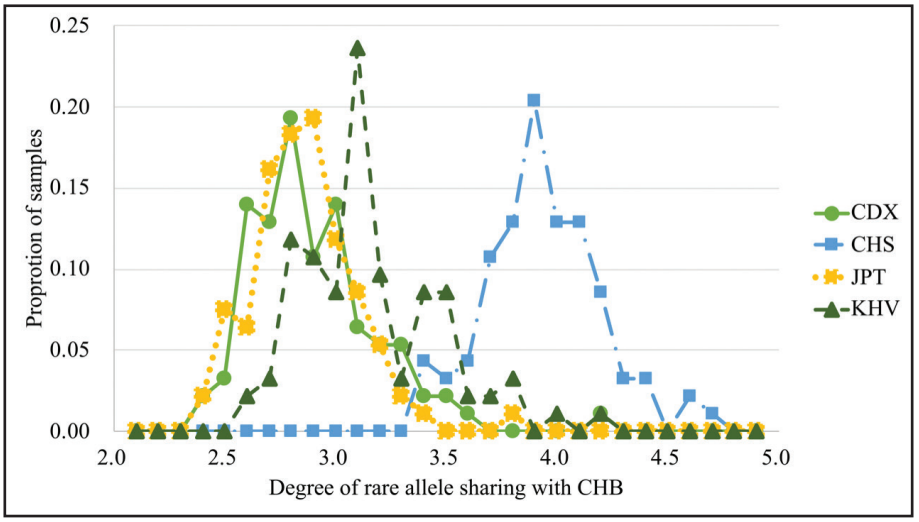

Fig. 1. *The degree of rare allele sharing with CHB normalized by that with YRI.*

The higher the degree of rare allele sharing with CHB the higher the sample affinity is to CHB. Among four test populations, CHS shows the highest degree of rare allele sharing with CHB. KHV is the second highest. JPT and CDX are the lowest of the four test populations and show similar degrees of rare allele sharing with CHB.

Comparison with genome wide F_{ST} values

According to the genome wide F_{st} values reported by 1KG, CHS has the smallest F_{st} to CHB (F_{st}=0.0011). CDX has the largest F_{st} to CHB (0.0086) among the four test populations. KHV and JPT have similar intermediate value of F_{st} to CHB (0.0062 and 0.0069, respectively) (The 1000 Genomes Project Consortium, 2015). The results of rare allele sharing follows the trend of genome wide F_{st} values except for JPT. In terms of genetic distance to CHB, JPT shows a similar value to KHV for F_{st}, but rare allele sharing shows that the result of JPT is close to that of CDX. One interpretation of this difference can be made based on the Dual structure hypothesis by Hahihara (1991). According to this hypothesis the Japanese population has two ancestries: Jomon and Yayoi migrants' components. It is also supposed that CHB shares a more recent common ancestor with Yayoi migrants and that CHB does not share recent ancestry with Jomon population (Kanzawa-Kiriyama, 2017). The Jomon components in JPT contributed a smaller degree of rare allele sharing with CHB and showed lesser amount of genetic affinity to CHB. Further, it is also possible that the contribution of Jomon components in JPT is not well captured or emphasized when genome wide F_{st} was used. If this is the case, rare allele sharing analysis can be applied to investigate the fine scale of Japanese population history.

Possible application of rare allele sharing on japanese population

Rare allele sharing can help reveal the variations of Yayoi migrant components among the regions of Japanese Archipelago. It is supposed that Yayoi migrants first reached North Kyushu of the Japanese Archipelago and gradually migrated toward the east (Miyamoto, 2019). This scenario suggests that the timing of admixture between indigenous Jomon people and Yayoi migrants in Japan varies from region to region. Then, the degree of Yayoi migrant ancestry should be different among local Japanese populations. In this paper, I examined JPT from the 1000 genomes project, these data cover only the Tokyo region. When the whole genome SNPs data become available for various

regions in the Japanese Archipelago, it can be tested whether there is a variation for the degree of admixture between Jomon and Yayoi migrants by investigating the degree of rare allele sharing with CHB. Furthermore, if such variation is observed, we might be able to trace the migration route of Yayoi migrants within the Japanese Archipelago. The populations located on or near to the route are expected to have higher degree of rare allele sharing with CHB than those located some distance from the route.

Application to ancient DNA: Yayoi period

During the Yayoi period, there should have been at least three different populations in the Japanese Archipelago in terms of their ancestry: 1. The indigenous Jomon population not yet subject to admixture with Yayoi migrants. 2. the Yayoi migrants themselves not yet subject to admixture with Jomon population, 3. the admixed population between Jomon and Yayoi population. This last admixed population is directly ancestral to the extant Japanese population. When DNA is extracted from human remains coming from Yayoi period archaic sites and whole genome sequencing is determined, we should be able to determine which of the three possible populations the individual belongs by investigating the degree of rare allele sharing with CHB. If the individual belongs to Jomon population, the degree of rare allele sharing with CHB should be smaller. If the individual belongs to Yayoi migrant population, the degree of rare allele sharing with CHB should be higher. When the individual shows an intermediate degree of rare allele sharing with CHB, he or she belongs to admixed population. By collecting various regions and various ages of ancient genomes from Yayoi period, we can then obtain an overview on how and when the Yayoi migrants spread over the Japanese Archipelago.

Application to ancient DNA: Jomon period

If multiple ancient genomes from Jomon period are collected, we should be able to have population data for Jomon population. Then we can investigate the degree of rare allele sharing directly with the Jomon population instead of with CHB. This will enable a more precise measurement of Jomon ancestry in individuals of current or ancient populations in the Japanese Archipelago. The Jomon period lasted for a long time. Therefore, we might predict that the Jomon population will have structural depth and might not be as homogeneous as Hanihara proposed. Then when we want to have population samples for the Jomon population, ideally it would be desirable that ancient

genomes are eventually collected with regards to a homogeneous age and geographical location.

Conclusions

I applied rare allele sharing analysis on the East Asians. By using CHB as the reference population and the other four population (CDX, CHS, JPT and KHV) as test populations, the overall genetic relationship among them were reproduced by the rare allele sharing analysis. The result of JPT is different from that of genome wide F_{st}. This difference can be explained by the Dual structure of Japanese which proposes that extant Japanese has two major ancestries, Jomon and Yayoi migrants. While Yayoi migrants are supposed to be genetically closer to CHB, Jomon has less genetic affinity to CHB. The Jomon component in JPT can cause the difference between rare alleles sharing and genome wide F_{st}. If this is the case, rare allele sharing can be used to reveal more a more detailed population history of the Japanese archipelago.

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Corresponding author: gojobori_jun@soken.ac.jp

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Population genomics on the origin of lactase persistence in Europe and South Asia

YOKO SATTA* NAOYUKI TAKAHATA*

Parole chiave: genomi umani antichi, hard selective sweep, costruzione di nicchia, 2D SFS.

Riassunto — La mutazione da C a T in rs4988235 del gene lattasi (*LCT*) è la determinante primaria nella persistenza di lattasi (LP) prevalente tra Europei e Sud Asiatici. Qui presentiamo un review degli studi evolutivi di questa mutazione basati su genomi umani antichi e attuali e concludiamo che la mutazione emerse nella Steppa Pontica tra 23.100 e 5.960 anni fa, arrivò in Europa e Sud Asia nell'Età del Bronzo attraverso l'espansione della popolazione ancestrale della Steppa, e fu sottoposta a *hard sweep* locali con insorgenze ritardate tra 5.000 and 3.280 anni fa. Precedentemente alla prima apparizione della mutazione di C a T, una mutazione da G ad A emerse in rs182549. L'aplotipo CA ancestrale all'aplotipo LP-related TA si trova ancora in campioni della Toscana, di Americani e Sud Asiatici mescolati, dove la grande maggioranza di discendenti con la mutazione di G ad A si spostarono, fino a che la mutazione da C a T fu favorita dalla selezione locale.

Key words: ancient human genomes, hard selective sweep, niche construction, Steppe ancestry, 2D SFS.

Summary — The C to T mutation at rs4988235 located upstream of the lactase (*LCT*) gene is the primary determinant for lactase persistence (LP) that is prevalent among Europeans and South Asians. Here, we review evolutionary studies of this mutation based on ancient and present-day human genomes and conclude that the mutation arose in the Pontic Steppe somewhere between 23,100 and 5960 years ago, arrived in Europe and South Asia in the Late Copper/Bronze Age via the expansion of the Steppe ancestral population, and exerted local hard sweeps with delayed onsets between 5000 and 3280 years ago. Prior to the first appearance of the C to T mutation, a closely linked G to A mutation arose at rs182549. The intermediate CA haplotype ancestral to the LP-related TA haplotype is still found in samples from Tuscans, admixed Americans and South Asians, whereas the great majority of G to A mutated descendants hitchhiked since the C to T mutation became favored by local selection.

Introduction

Most mammals lose the ability to digest lactose after weaning, but some present-day humans continue to express the key digestion enzyme, lactase (*LCT*), in the small intestine throughout adult life

* School of Advanced Sciences, SOKENDAI (The Graduate University for Advanced Studies), Hayama, Kanagawa 240-0193, Japan.

(Enattah *et al*., 2002; Ségurel and Bon, 2017). This physiological change is referred to as lactase persistence (LP), and has attracted much attention particularly from the viewpoint of gene-culture coevolution, human self-domestication or niche construction (e.g., Aoki, 1986; Itan *et al*., 2009, 2010; Gerbault *et al*., 2011, 2013; O'Brien and Laland, 2012). Apart from LP in Africa (Ingram *et al*., 2007; Tishkoff *et al*., 2007), LP in Eurasia is tightly associated with derived alleles T at rs4988235 and A at rs182549 of single nucleotide polymorphisms (SNPs) that are located -13,910 bp and -22,018 bp, respectively, upstream of the *LCT* gene. The association of the C/T polymorphism at rs4988235 with non-LP/LP is complete in Finnish and non-Finnish (Korean, Italian and German) samples, and that of the G/A polymorphism at rs182549 is nearly complete in case-control study samples (Enattah *et al*., 2002). Both the T and A alleles enhance the *LCT* promoter activity, although the A allele results in minimal enhancement compared with the T allele (Olds and Sibley, 2003; Troelsen *et al*., 2003; Lewinsky *et al*., 2005).

Aside from the adaptive significance, the dating of LP or T allele origin in Europe has received significant interest in light of ancient genomes. Using early Holocene human remains, Burger *et al*. (2007) showed that most Meso- and Neolithic Europeans lacked the T and A alleles, concluding that LP arose in the last 20,000 years – hereafter denoted as $t_{LCT} < 20{,}000$ (Bersaglieri *et al*., 2004; Coelho *et al*., 2005; Leonardi *et al*., 2012). Lack of the T allele in European Neolithic farmers was directly demonstrated by the Chalcolithic Tyrolean Iceman, a 5300-year-old natural mummy discovered in the Ötztal Alps (Keller *et al*., 2012) as well as a 7400-year-old Cardial individual from Cova Bonica in Barcelona (Olalde *et al*., 2015). Obviously, however, more individuals were needed to confirm this absence. A recent study of 400 Neolithic, Chalcolithic and Bronze Age Europeans (Olalde *et al*., 2018) observed that the T allele remained at a very low frequency across the transition from the Neolithic period to the Bell Beaker and Bronze Age periods, both in Britain and continental Europe, with a major increase in its frequency occurring only within the last 3500 years (Cassidy *et al*., 2016; Brace *et al*., 2018). Tab. 1 summarizes some such results of Allentoft *et al.* (2015), Mathieson *et al.* (2015), and Olalde *et al.* (2018) based on the Central European chronology in Haak *et al.* (2015); readers may further refer to Witas *et al.* (2015), Liebert *et al.* (2017) and Ségurel and Bon (2017) together with Ségurel *et al.* (2020) and Jeong *et al.* (2020) both of which address an enigmatic 5000-year history of lactose adaptation in Central and Eastern Asian herders.

Central Europe	Cultures in Europe and Steppe	a	b	c
Paleolithic	Pleistocene hunter-gatherer	0	-	0
43,000-10,000 BC				
Mesolithic	Mesolithic hunter-gatherer	0.17 [e]	0	0
9700-6200 BC	8.2 event [d]			
Early Neolithic	Holocene hunter-gatherer	0	0	0
6000-4000 BC	LBK (5500 -4800BC)			
Middle Neolithic	Late Copper Age	0	0	0
4000-3000 BC	Yamnaya (3000-2400 BC)			
Late Neolithic	CWC (2800-2300 BC)	~0.20		
2600-2200 BC	BBC (2600-2000 BC)		~0.05	~0.05 [f]
Bronze Age	Sintashta (2100-1800 BC)	0.05	~0.05	-
2200-1000 BC	Andronovo (1700-1500 BC)			
Iron Age 900 BC		-	-	~0.05 [f]
Modern Europeans [g]		0.67	0.74	0.46

Tab. 1. *Frequencies of the T allele at rs4988235 in ancient and present-day genomes in Central Europe. a, Allentoft et al. (2015); b, Mathieson et al. (2015); c, Olalde et al. (2018); d, time of an abrupt climate change caused by a glacier meltwater outburst 8200 years ago; e, after Fig. 4a and Supplementary Tab. 1, but 0 when inferred from imputation of ancient genomes (Fig. 4b); f, extended data Fig. 7 in Olalde et al. (2018); g, North Europe, CEU, and IBS, respectively. The chronology of Central Europe (left) is taken from Haak et al. (2015).*

The geographic location of the origin of European LP was vague and contentious (Enattah *et al.*, 2002, 2007 for the Pontic Steppe or Caucasian origin; Itan *et al.*, 2009 and Gerbault *et al.*, 2011 for theoretical modeling of LP/dairying coevolution). This uncertainty is because the location of origin does not necessarily coincide with the current geographic center of the LP distribution (Edmonds *et al.*, 2004). Gerbault *et al.* (2011) provided an interpolated map of the distribution of the T allele in the Old World and summarized simulation results for the spread of European LP in time and space. Indeed, the T allele frequency varies considerably even within Europe (the 1000 Genomes Project Consortium, 2015; subsequently, we abbreviate the sequence dataset as 1000GD): 74% in Northern Europeans from Utah (CEU), 59% in Finnish from Finland (FIN), 72% in British from England and Scotland (GBR), 46% in Iberians from Spain (IBS), and 8.9% in Tuscans from Italy (TSI), with the overall frequency being 511/1006=51% in the European meta-

population (EUR).

To gain some additional insights into these problems regarding LP or the T allele at rs4988235 and the A allele at rs182549, we separately applied a new inference method (Satta *et al.*, 2019) to an *LCT* region of 100 kb for the five individual European populations and one South Asian population (PJL: Punjabi from Lahore) in the 1000GD (Smith *et al.*, 2018). We summarize and discuss results of the application together with a phylogenetic analysis of *LCT* haplotypes in comparison with recent ancient genomic studies. Further, we demonstrate that one variable used in the method can pinpoint rs4988235 as the causative mutation for LP.

Inference of a selective sweep under nonequilibrium demography

To detect, classify and date an incomplete selective sweep for *LCT* in each population, we used the method of the two-dimensional site frequency spectrum $\{\varphi_{i,j}\}$, 2D SFS, by Satta *et al.* (2019). This method first seeks for a region under strong linkage disequilibrium (LD with $r^2 > 3/4$) surrounding a target site and divides a sample of size n into the derived and ancestral allele groups defined by the site. Each element of the $\{\varphi_{i,j}\}$ matrix then counts the number of segregating sites or SNPs within the region at each of which we find exactly i and j derived alleles in the derived and ancestral allele groups, respectively. It is to be noted that $\varphi_{i,j} > 0$ for possible positive i and j stems most likely from recombination between the two allele groups, although it is expected to be rare in a tightly linked region.

We compare the performance of our inference method with that of recent studies. Such studies include Itan *et al.* (2009) who estimated $6256 < t_{LCT} < 8683$ using an approximate Bayesian computation (ABC) approach, Field *et al.* (2016) who developed the singleton density score method and estimated $t_{LCT} \geq 2000$ from the UK10K project, Smith *et al.* (2018) who developed a hidden Markov model and obtained t_{LCT}= 9341 (95% CI: 8688-19,989) for IBS, 6869 (5143-8809) for BEB (Bengali in Bangladesh) and 9514 (8596-10,383) for PJL, Harris *et al.* (2018) who based their method on haplotype structure, Akbari *et al.* (2018) who claimed the high power of their iSAFE method to pinpoint the causative mutation of a single sweep, Nakagome *et al.* (2019) who developed an ABC method conditioned on the allele frequency in the past and applied it to four pigmentation SNPs, Satta *et al.* (2019) who used the 2D SFS method for EUR and estimated t_{LCT}=3280±480, and Tournebize *et al.* (2019) who obtained t_{LCT}=4250 (95% CI: 3700-17,680) for

CEU. Readers may also refer to Smith *et al.* (2018) and Tournebize *et al.* (2019) for various estimates of t_{LCT} either as the time to the most recent common ancestor (TMRCA) or as the age of the allele (Tishkoff *et al.*, 2007; Itan *et al.*, 2009; Nakagome *et al.*, 2016).

Here, we focus on the TMRCA within the derived allele group because our interest is in the time (t_{SEL}) of onset of positive selection rather than the origination time (t_{AGE}) of a mutation that produced the allele. Hereafter, we use the symbol t_{LCT} to denote the TMRCA of the derived allele group. Obviously, t_{AGE} is no shorter than t_{LCT}, and t_{SEL} is no greater than t_{AGE}, although none of these times is directly observable. For instance, to infer t_{SEL}, Enattah *et al.* (2007, 2008) had to assume the initial frequency of the T allele and the selection intensity in a simple equation of describing an allele frequency change. Nevertheless, the distinction between t_{LCT} and t_{SEL} is important with respect to the mode of selective sweep. A necessary condition for soft sweeps (Hermisson and Pennings, 2005, 2017) is $t_{LCT} > t_{SEL}$. This is equivalent, though not exactly, to saying that multiple distinct ancestral lineages carrying a common mutation simultaneously undergo positive selection. An obvious caution is that positive selection acting on a standing variation does not necessarily classify the sweep as soft. To the contrary, a sufficient condition for hard sweeps is $t_{LCT} \leq t_{SEL}$. There are thus two distinguishable cases: hard ($t_{LCT} \leq t_{SEL} \leq t_{AGE}$) and *conditional* soft sweeps ($t_{SEL} < t_{LCT} \leq t_{AGE}$), the latter being related to the hardening of soft sweeps (Wilson *et al.*, 2014). It seems that either origin of a target mutation or population structure is irrelevant or even confusing to this distinction.

To evaluate statistical significances of our estimates, we assumed a demographic model and neutrality of genome evolution (Kimura, 1968). We used a very simplified bottleneck and expansion model that incorporates some aspects of human nonequilibrium demographic history inferred thus far by SFS (Schaffner *et al.*, 2005), PSMC (Li and Durbin, 2011), MSMC (Schiffels and Durbin, 2014), Stairway Plot (Liu and Fu, 2015), and SMC++ (Terhorst *et al.*, 2017) among others. In our simplified model, a single bottleneck is shared by all non-African populations from the exodus out of Africa (ca. 58,000 years ago) to the initial Eurasia-wide dispersal (ca. 45,000 years ago), and a population had since grown exponentially up to the population size 500 years ago (Fig. 1, Tab. 2a). We assumed the size of the bottlenecked population to be 2000 and ignored the effect of population explosion during the last 500 years. The robustness of the method to nonequilibrium demography was discussed in Satta *et al.* (2019). For genome evolution, we assumed the infinite-sites model (Kimura, 1969), but with linkage.

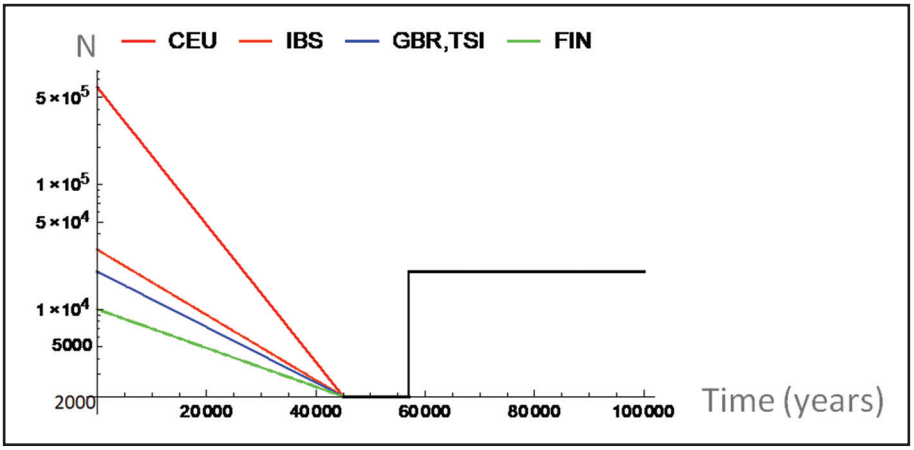

Fig. 1. *Demographic models for five European populations.*
The effective population size (Y axis in log scale) is depicted against the past time (X axis in years). Four colored lines show population-specific demographic models with different exponential growth rates (α) (Tab. 2a). The models assume that the size (500 years ago) was 600,000 for CEU, 30,000 for IBS, 20,000 for GBR and TSI, and 10,000 for FIN. All populations began to expand 45,000 years ago after experiencing a bottleneck phase of constant size 2000 since this ancestral Eurasian population migrated out of Africa 58,000 years ago. The ancestral African population size was 20,000.

Population	CEU	FIN	GBR	IBS	TSI
α	1.3×10^{-4}	3.6×10^{-5}	5.1×10^{-5}	6.0×10^{-5}	5.1×10^{-5}
m/n	146/198	117/198	131/182	98/214	19/214
S_ξ	213	218	204	287	351
F_c	0.0141	0.0076	0.0135	0.0049	0.0059
(P)	($< 10^{-4}$)	($< 10^{-4}$)	($< 10^{-4}$)	($< 10^{-4}$)	(0.015)
L_{c0}	0.0026	0.0021	0.0026	0.0017	0.0002
(P)	($< 10^{-4}$)	($< 10^{-4}$)	($< 10^{-4}$)	($< 10^{-4}$)	(0.013)
G_{c0}	1.09	1.25	1.16	1.50	1.00
(P)	($< 10^{-4}$)	($< 10^{-4}$)	($< 10^{-4}$)	($< 10^{-4}$)	(0.036)
C	0.164	0.171	0.168	0.184	0.105
(S. E.)	(0.036)	(0.047)	(0.042)	(0.066)	(0.074)

Tab. 2a. *IAV variables in the 100 kb LD region surrounding rs4988235 in five European populations in the 1000GD (sample size n and derived allele frequency f_r=m/n).The P values in parentheses were computed from simulation results of 10^4 replications of ms (Hudson, 2002) under the bottleneck and exponential growth model with the per-year rate α (Fig. 1). The variables are defined as* $F_c = \frac{\Sigma i\varphi_{i,j}}{\Sigma(i+j)\varphi_{i,j}}$, $L_{c0}=L_{\zeta 0}/L_\xi$, $G_{c0}=L_{\zeta 0}/S_{\zeta 0}$ *and* $C=L_{\zeta 0}/m$ *where* Σ *in* F_c *indicates that the summation is taken over a certain range of i and j (see Fujito et al., 2018 and Satta et al., 2019 for details),* $S_{\zeta 0} = \sum_{i=1}^{m-1} \varphi_{i,0}$, $L_{\zeta 0} = \sum_{i=1}^{m-1} i\varphi_{i,0}$, $S_\xi = \sum_{i=0}^{m}\sum_{j=0}^{n-m} \varphi_{i,j}$ *and* $L_\xi = \sum_{i=0}^{m}\sum_{j=0}^{n-m}(i+j)\varphi_{i,j}$.

Results

The hallmark of an incomplete selective sweep is reduced intra-allelic variability (IAV) within a derived allele group relative to the total variability in the entire sample (Fujito *et al.*, 2018; Satta *et al.*, 2019). IAV can be expressed by numerous variables that are useful for detecting and classifying a selective sweep as well as for dating the onset of positive selection. The significance test for individual variables F_c, L_{c0} and G_{c0} used to measure the level and pattern of IAV shows that the observed values are as extreme as 10^{-4} in all but the TSI population (Tab. 2a). To further strengthen the test, we combined these mutually correlated variables, but to avoid inflating false positives, we used the method by Brown (1975) that combines non-independent, one-sided tests of significance (Poole *et al.*, 2016). The results of this test show that, in the European populations including TSI, the individual IAVs become highly incompatible with neutrality (Tab. 2b).

Population	CEU	FIN	GBR	IBS	TSI
$\rho(F_c \cdot L_{c0})$	0.383	0.539	0.470	0.551	0.594
$\rho(F_c \cdot G_{c0})$	0.124	0.422	0.132	0.560	0.357
$\rho(L_{c0} \cdot G_{c0})$	0.535	0.532	0.503	0.591	0.497
χ_f^2	34.1	29.1	33.3	27.1	13.4
(c) (f)	(1.62) (3.7)	(1.90) (3.2)	(1.66) (3.6)	(2.04) (2.9)	(1.88) (3.2)
Combined P	$< 3 \times 10^{-7}$	$< 3 \times 10^{-6}$	$< 7 \times 10^{-7}$	$< 5 \times 10^{-6}$	0.005

Tab. 2b. *P values obtained by the method that combines non-independent, one-sided F_c, L_{c0} and G_{c0} variables. The combined variable is given by $X^2 = \sum_{l=1}^{k}(-2\ lnP_l)$ where P_l is the probability that the l-th variable exceeds the observed value under the null hypothesis. The minimum value of X^2=55.3 corresponding to the three maximum P values of 10^{-4} (Tab. 2a) is used for the first four populations. $X^2/c=\chi_f^2$ is approximated by a chi-square variable with f degrees of freedom, provided that c and f are computed from the correlation coefficient ρ=ρ(Y·Z) between variables Y and Z. Brown (1975) fitted a quadratic function of ρ to covariance Cov(-2lnP$_l$,-2lnP$_l$'), whereas Kost and McDermott (2002) fitted a third-order polynomial (Poole et al., 2016). Except for two cases between F_c and G_{c0}, ρ is as high as 0.6; yet, the combined method rejects the null hypothesis of neutrality at a level of significance of at most 0.005 (TSI).*

The 2D SFS method then infers that the mode of the selective sweep by the T allele is definitely hard. In addition to unusually reduced IAV levels measured by F_c and L_{c0}, this hardness is captured as low

levels of multiplicity (G_{c0}) of derived alleles per SNP (Tab. 2a). Indeed, almost all alleles per SNP are singletons or doubletons, irrespective of populations, such that G_{c0} is close to 1 to 2. No other target sites that exhibit such low multiplicity in such a wide genomic region have yet to be revealed (Satta *et al*., 2019). The 2D SFS method also allows us to estimate the mutation-rate-scaled TMRCA (ut_{LCT}) from the total number ($C = \sum_{i=1}^{m-1} i\varphi_{i,0}/m$) of derived alleles per sample within the T allele group of size $m < n$. It is to be noted that recombinant SNP sites are automatically excluded from C. Using $ut_{LCT}=C$ or $t_{LCT}=C/u$ (Tab. 2a), we have t_{LCT}=3280±720 in CEU of m=146, 3420±940 in FIN of m=117, 3360±840 in GBR of m=131, 3380±1320 in IBS of m=98, and 2100±1480 in TSI of m=19, if the above mutation rate (u) is 5×10^{-5} per 100 kb per year (Scally and Durbin, 2012).

We also conducted phylogenetic analyses to show that almost all T haplotypes (haplotypes within the T allele group defined in the LD region), including those in TSI and PJL, are tightly clustered so as to form a single clade (Fig. 2). Unexpectedly, there is one T haplotype (HG03653) in PJL that is clustered in the ancestral C allele group. The DNA sequence of HG03653 suggests three possibilities: a mistyping of C to T at rs4988235, a parallel mutation in a divergent haplotype background resulting in the TG haplotype, or a migrant of the TG haplotype from the Urals or the Caucasus as discussed below. In general and more importantly, the ancestral C haplotype closest to the T cluster differs only by a single C to T mutation. This result confirms the previous conclusion that the T haplotypes were descended from an ancestral C haplotype in the recent past by a single mutation (Enattah *et al*., 2007, 2008).

It is also interesting to examine how accurately some of our variables can pinpoint a true target site of positive selection (Fig. 3). For this purpose, we selected F_c based on the fact that recombination plays essential roles in pinpointing a target site and that F_c includes recombinant SNPs, whereas both L_{c0} and G_{c0} do not. Nominating all candidate SNPs in a 2.5 Mb genomic region surrounding *LCT*, we measured F_c for each candidate within a window of 300 SNPs and then ranked all of these F_c values in the whole 2.5 Mb region. We also compared the result with that by the iSAFE with an «inserted» procedure (Akbari *et al*., 2018), and found that this procedure does not improve the power of F_c. Most importantly, like iSAFE, F_c ranked the T allele at rs4988235 as 1 (and the A allele at rs182549 as 2).

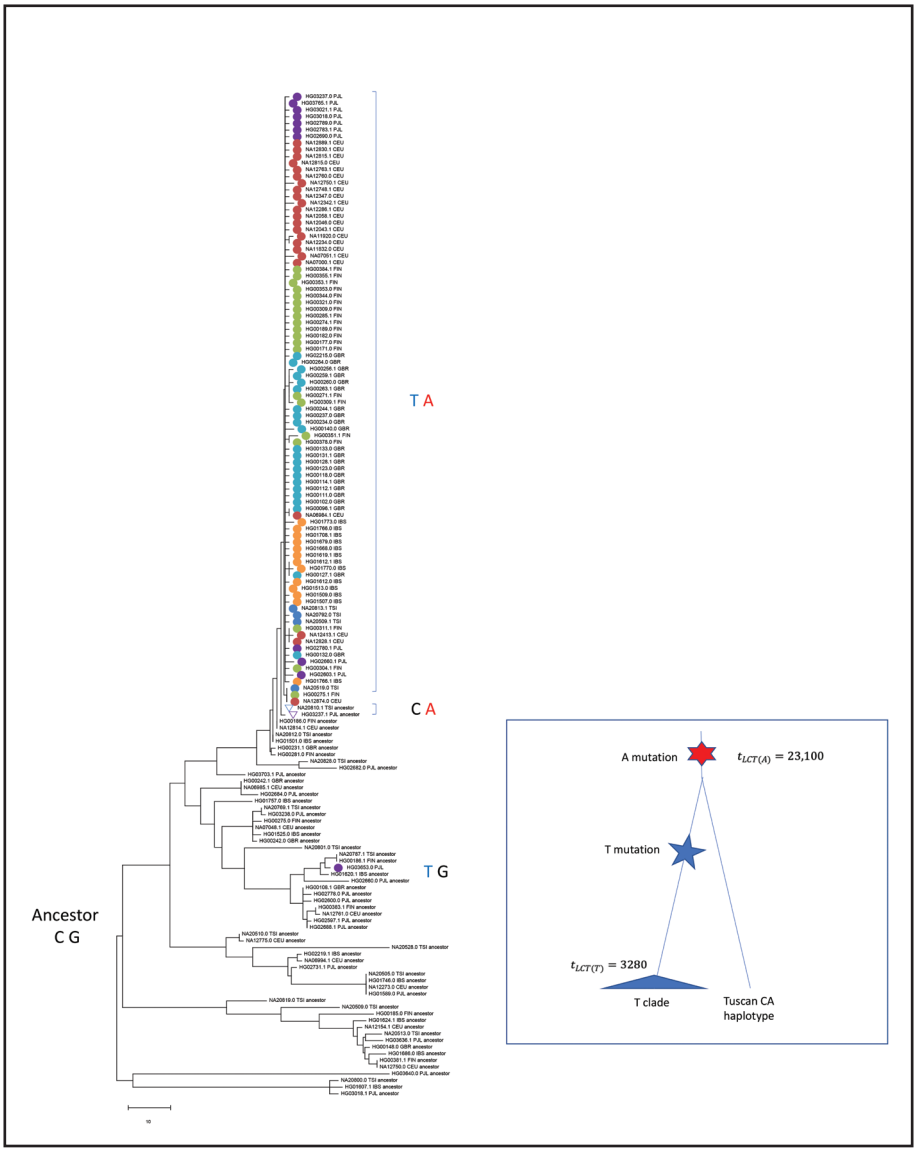

Fig. 2. *Unrooted NJ tree for a subsample of the T and C distinct haplotypes. The T/C sample size is 23/9 from CEU, 13/9 from IBS, 22/5 from GBR, 4/12 from TSI, 20/7 from FIN, and 11/15 from PJL. The LD region in each population is the same as that in the entire EUR sample (Satta et al., 2019). TA haplotypes at rs4988235 and rs182549 are marked by filled colored circles and CA haplotypes in TSI and PJL by two open inverse triangles. The tree was constructed from pairwise nucleotide differences without multiple-hit correction (Saitou and Nei, 1987) in which the scale bar represents 10 nucleotide differences per 100 kb. The inset is a cartoon of the haplotype evolution. The G to A mutation occurred > 23,100 years ago, the C to T mutation emerged much later in an A lineage and swept through a population. The CA haplotype in TSI and PJL is a remnant of the A mutation that escaped from the selective sweep.*

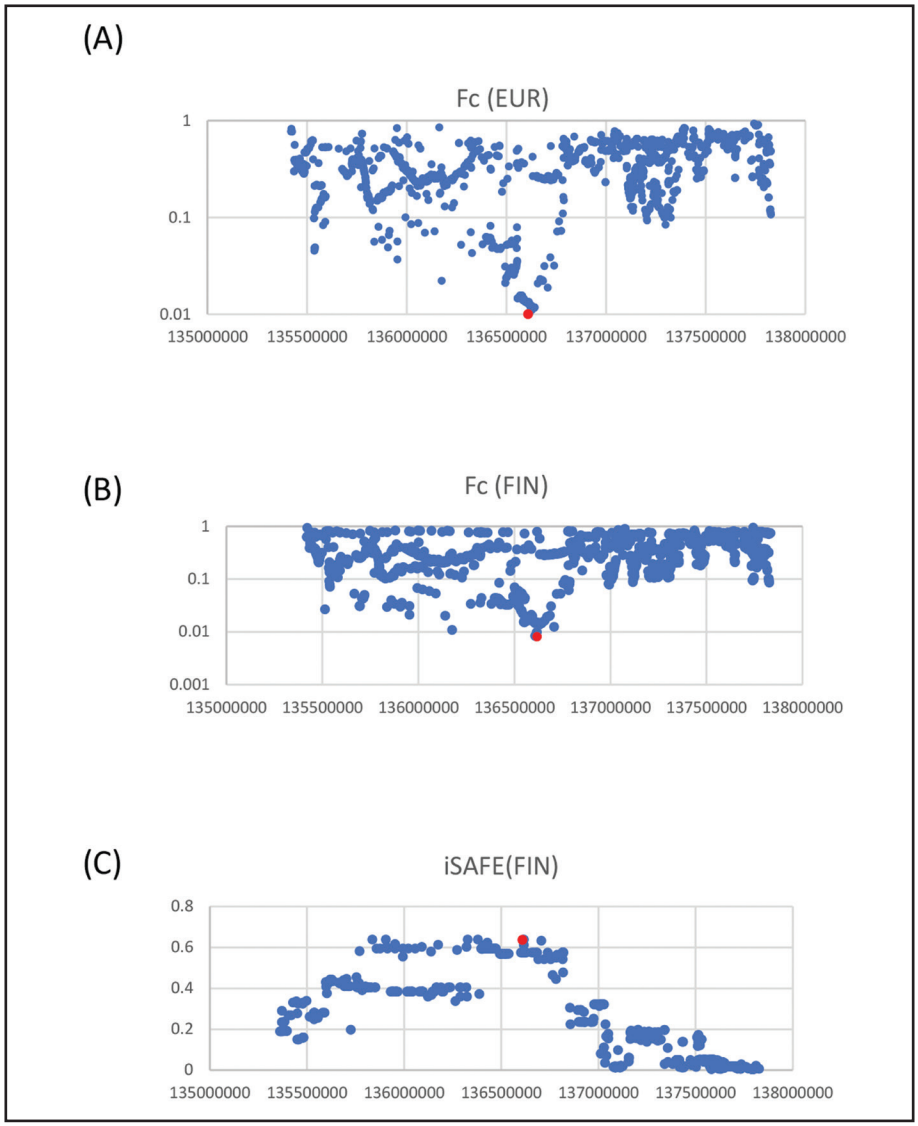

Fig. 3. *Identification of a target site of selection.*
We searched for a target site from candidate SNPs surrounding LCT (X axis for genomic positions) by ranking their F_c and iSAFE values (Y axis). In F_c, candidate SNPs were all derived alleles whose frequencies are > 45% in EUR and > 54% in FIN. Note that, although derived alleles at low frequencies were excluded from candidates, they were used to compute F_c. The red points indicate the F_c or iSAFE value for the T allele at rs4988235. Needless to say, the ranking by F_c is in ascending order (Fujito et al., 2018), whereas that of iSAFE is in descending order (Akbari et al., 2018). (A), (B) F_c scanning over the 2.5 MB genomic region in the EUR sample of size n=1006 and the FIN sample of size n=189; (C) iSAFE scanning over the 2.5 MB genomic region in the FIN sample.

Discussion

Farming was introduced to most of Europe from Anatolia/Levant by 6000 to 5000 BC via two main routes: the Danube river line and the northern Mediterranean coast line. Farmers in Central Europe and Iberia were respectively associated with the LBK (Linearbandkeramik) tradition along the river line and the *Impresso*-Cardium culture along the coast line (Olalde *et al.*, 2015; Lazaridis *et al.*, 2016; Hofmanová *et al.*, 2016; Broushaki *et al.*, 2016; Brace *et al.*, 2018; Mathieson *et al.*, 2018; Souilmi *et al.*, 2020). In each route, farming was brought upon by demic diffusion with limited admixture from local hunter-gatherers and accordingly an apparent decrease in the Neolithic contribution with geographic distance from the Near East (Bramanti *et al.*, 2009; Skoglund *et al.*, 2014; Günther *et al.*, 2015). Indeed, while Scandinavian farmers were intimately related to farmers in Southern Europe, such as the Tyrolean Iceman and Sardinians, they exhibited high levels of admixture from local hunter-gatherers (Skoglund *et al.*, 2012, 2014). Rivollat *et al.* (2020) also revealed a higher proportion of western hunter-gatherer ancestry in Western European farmers than in Central European farmers, pointing to the complexity of their interactions in France where both routes converged.

Olalde *et al.* (2018) showed that Neolithic Anatolian and Aegean farmers were indeed non-LP despite archaeological and genetic evidence for their taurine cattle domestication and milk use around 6500 BC (Evershed *et al.*, 2008; Curry, 2013). Allentoft *et al.* (2015) found the T allele at low frequency in the Late Copper/Bronze Age of Eurasia (ca. 3000-1000 BC) and showed a possible Steppe origin of LP from the highest occurrence of the T allele in the Yamnaya and Corded Ware cultures (CWCs) between 3000/2800 and 2300/2000 BC. Haak *et al.* (2015) also observed that the T allele increased in frequency only after the Yamnaya Steppe ancestry became ubiquitous in Central and Western Europe. Consistent with these findings, it was recently identified that the T allele is possessed by only one CWC individual from Sweden (Malmström *et al.*, 2019), only one Final Neolithic individual from Switzerland (Furtwängler *et al.*, 2020), and none of 16 CWC individuals from Poland (Witas *et al.*, 2012; Linderholm *et al.*, 2020), to mention a few. Using 230 ancient Eurasians, Mathieson *et al.* (2015) found that the earliest appearance of the T allele is in a Central European Bell Beaker sample dated to between 2450 and 2140 BC, and the selective sweep dated to the last 4000 years (Burger *et al.*, 2007; Gamba *et al.*, 2014; Haber *et al.*, 2016). Bell Beaker culture (BBC) was widespread in Western and

Central Europe from 2750/2500 to 2200/1800 BC, and associated with Steppe-related ancestry with the replacement in gene pool being most pronounced in Britain (Olalde *et al.*, 2018). Interestingly, however, Northern Italy and Sicily Bell Beakers had no sign of LP. Raveane *et al.* (2019) and Fernandes *et al.* (2020) pointed out that they can be modeled certainly as Anatolian-farmer-related, though with different affinities to Steppe Bronze Age. Olalde *et al.* (2018) also argued that there is limited genetic affinity between Beaker-complex-associated individuals from Iberia and Central Europe. Furthermore, focusing on the 8000-year history of Iberia, Olalde *et al.* (2019) concluded that, in Iberia, the T allele continued to occur at low frequency in the Iron Age, pointing to recent strong positive selection; however, they also showed that present-day non-Indo-European speaking Basques inherited substantial levels of Steppe ancestry, consistent with Late Neolithic Basques with the 27% LP-associated T allele frequency (Plantinga *et al.*, 2012). In short, although estimates of the ancient T allele frequency still vary, neither hunter-gatherers nor early farmers in Anatolia and Europe possessed the T allele (Tab. 1) and the earliest carriers came from CWC and BBC individuals in Central Europe, Iberia and Scandinavia after ca. 2500 BC.

It is therefore reasonable to assume that the T allele arose somewhere in the Pontic Steppe and emigrated into Central Europe via the Yamnaya expansion in ca. 3000 BC. Yet, positive selection began to operate for the immigrated T allele somewhat later. As positive selection acted on such a standing variation ($t_{SEL} < t_{AGE}$), the selective sweep might be defined as soft. Nonetheless, the level and pattern of IAV in the T allele group are best described as hard. In this context, it is not really important to distinguish between hard and soft sweeps based on the origin of a selected mutation. We rather classify the mode based on IAV in a linked genomic region. The delayed onset of positive selection for European LP suggests the initial operation on genetically indistinguishable T haplotypes ($t_{LCT} \leq t_{SEL}$). This results from either where selection operated on a single ancestral lineage or where multiple descendants, if involved, did not have enough time to differentiate from each other by mutations. In either event, both cases lead to $t_{LCT} \leq t_{SEL} < t_{AGE}$ and look like hard sweeps. Evaluation of t_{AGE} can be directly made from the age estimate of the T allele, but here we used the TMRCA ($t_{LCT(A)}$) of the derived A allele group defined at rs182549 as a surrogate of the age of the T allele ($t_{AGE(T)}$). The widespread T allele occurred in the background of the A allele and $t_{AGE(T)}$ is bounded by $t_{LCT(A)}$=23,100±20,000 in EUR. Thus, $t_{AGE(T)}$ must be somewhere between 23,100 and 3280. Recently, examining

the currently available ancient genomes, Ségurel *et al.* (2020) found that the earliest appearance of the T allele was in Ukraine 5960 years ago (Mathieson *et al.*, 2018). With this find, $t_{AGE(T)}$ becomes older than 5960 years ago. On the other hand, as $t_{LCT(A)}$ is well after non-African populations differentiated from each other, it is likely that both the T and A alleles originated in a single locally differentiated Eurasian population. However, there is a curious exception to this conclusion. From genotyping a ~30 kb *LCT* region in > 1600 samples from 37 global populations, Enattah *et al.* (2007) found that the T mutation occurred at least twice independently on highly divergent haplotype backgrounds and that one mutation is geographically restricted to populations living in west of the Urals and north of the Caucasus. We caution that a similar mutation resulting in the TG haplotype is found in PJL (Fig. 2) and that its inclusion into the T allele group increases the TMRCA, although the extent depends sensitively on estimation methods used. In the Atlas of Variant Age developed by Albers and McVean (2020), the age of the T allele, including the TG haplotype, is estimated as 693 generations or ca. 20,000 years if the generation time is 29 years.

The Tuscans represent an interesting caveat. Clearly, the present-day TSI is an outlier in terms of European LP, exhibiting an exceptionally low T allele frequency in a large sample. By comparing TSI genomes with other Europeans and Middle Easterners, Pardo-Seco *et al.* (2014) revealed that admixture took place between 1100 and 600 BC. Although this admixture event may have been much older and even recurrent, it implies the partial eastern origin of non-Indo-European speaking Etruscans, ancestral to Tuscans in the Bronze/Iron Age. Intriguingly, some cattle breeds (*Bos taurus*) were also simultaneously imported from the east Mediterranean area (Pellecchia *et al.*, 2007). However, the T allele in TSI suggests that this cattle import did not act as a selective agency for LP due presumably to lack of relevant culture or absence of the T allele *per se* at that time. We emphasize that, although evidence for positive selection is relatively weak for TSI, the Brown's combining method could detect a hard sweep by the T allele (Tab. 2b). One possibility is that the T allele in TSI resulted from recent gene flow from neighboring LP areas (Fiorito *et al.*, 2015 for gene flow within Italy). Coelho *et al.* (2005) estimated the LP frequency to be 24% and the T allele frequency to be 13% in Central Italy (37 individuals from Tocco da Causaria and 30 from Rome). While these frequencies in Central Italy are much lower than those in Portugal, they are much higher than those in Tuscany; if statistically tested, Central Italian genomes would undoubtedly show a hard sweep.

TSI is exceptional also in terms of pairwise LD between C/T at rs4988235 and G/A at rs182549. While the TA haplotype at these SNPs is in complete LD (r^2=1) in CEU, FIN, GBR, and IBS, it is not (r^2=0.95) in TSI, owing to the presence of a CA haplotype in addition to derived TA and ancestral CG haplotypes in the 1000GD (Fig. 2). As a number of CA haplotypes can be also found in MXL (Mexican Ancestry from Los Angeles) and PEL (Peruvian in Lima) from admixed Americans (AMR), as well as PJL, BEB, ITU (Indian Telugu in the UK), and STU (Sri Lankan Tamil from the UK) from South Asian Ancestry (SAS), the age of the A allele must be older than that of the T allele as aforementioned. Interestingly, the T allele frequency is quite high in some of these populations: 24% in MXL, 11% in PEL, and 26% in PJL. Yet, IAV is substantially reduced in MXL or even non-existent in PEL (i.e., t_{LCT}=0). These features are compatible with very recent ongoing selective sweeps, but more likely causes would be admixture, founder effects, or a combination of these factors after Columbus. Similarly, IAV in PJL is significantly reduced (χ_f^2=23.3 with c=2.01 and f=2.98; P=4×10^{-5}) with t_{LCT}=3600±1327 or 3265±1291, depending on the inclusion or exclusion of the TG haplotype (Fig. 2). Although admixture also occurred in PJL, we consider a prehistoric event as a more likely cause: if the T allele arose in the Pontic Steppe, it must have spread not only into Europe from 3000 BC, but also into Turan by 2100 BC and further into South Asia after the decline of the Indus Valley Civilization in ca. 1800 BC (Damgaard *et al*., 2018; Narasimhan *et al*., 2019; Shinde *et al*., 2019). As the time elapsed in Punjab was as long as 3800 years, it is tempting to speculate the presence of an independent selective sweep by the T allele in this locality too. Thus, it appears that LP in Europe and South Asia shares the early history of the expanding Steppe ancestry in the Bronze Age, and offers strong selective advantages to its local bearers in lactose-relevant niche construction.

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Note added in Proof — After this paper had been sent to press, Burger *et al.* (2020) published a paper in *Current Biology*, 30: 1-9 that enriches the content of Tab. 1 concerning T allele frequencies in various post-Neolithic population samples.

Corresponding author: Yoko Satta: satta@soken.ac.jp

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Anthropological viewpoint gains insights into the transmission mode of Epstein-Barr virus

GENTO TADAKARA* HANA SHIMIZU-FURUSAWA**
AUGUSTINUS SOEMANTRI*** VANNARAT SAECHAN****
WANNAPA SETTHEETHAM-ISHIDA*****
TAKAFUMI ISHIDA*

Parole chiave: EBV, percorso di transmissione, diversità genomica, residenza matrilocale e patrilocale.

Riassunto — Il virus Epstein-Barr (EBV), uno dei linfocriptovirus (LCV), è un virus onnipresente, con tassi di prevalenza elevati nella maggior parte delle popolazioni umane. I portatori EBV depositano le particelle del virus nella loro saliva e la trasmissione può avvenire attraverso la saliva. Per quanto riguarda le forme di trasmissione primaria di EBV, è stata proposta ed è ora generalmente accettata l'ipotesi che EBV si trasmetta da madre a figlio nei primi stadi della vita. Tuttavia dati più precisi per testare il contributo materno alla trasmissione di EBV rimangono sfuggenti. Per fare luce su questo problema sono state applicate due strategie: 1) confronto diretto del genoma EBV tra madre e neonato, e 2) testare la diversità genomica di EBV in comunità matrilocali e patrilocali. Sequenze di Nucleotidi per EBV-*LMP1* sono stati ottenuti da 53 coppie madre-figlio (residenti di Sumba Island, Indonesia). Il contributo materno alla trasmissione di EBV era minore della metà. Una sequenza identica condivisa da madre e figlio è stata individuata in 18 coppie (34.0%), mentre in 35 coppie (66.0%), sono state trovate sequenze nucleotidiche differenti. Un totale di 189 sequenze del gene EBV *LMP1* proveniva da residenti etnici nel nord della Thailand: 100 da gruppi patrilocali e 89 da gruppi matrilocali. La diversità nucleotidica (π) è stata calcolata in 0.023 ~ 0.037 nei gruppi patrilocali e 0.024 ~ 0.038 in quelli matrilocali. La diversità genomica EBV non differisce fra i gruppi patrilocali e matrilocali ($p > 0.05$). Questi due approcci mostrano che le madri non contribuiscono necessariamente all'infezione primaria EBV nei primi stadi di vita.

Key words: EBV, transmission route, genome diversity, matrilocal and patrilocal residence.

Summary — Epstein-Barr virus (EBV), a member of the lymphocryptovirus (LCV), is an ubiquitous virus with high prevalence rates in most human populations. EBV carriers shed the virus particles into their saliva and transmission via saliva can occur. As for the primary EBV transmission, a hypothesis that EBV transmits from mother to child in the early stage of life has been proposed and up to now generally accepted. However, more precise data to test the maternal contribution to the EBV transmission remains elusive. To shed light on this issue, two approaches were employed; 1) direct comparison of the EBV genome between mother and infant, and 2) testing EBV

* Department of Biological Sciences, School of Science, University of Tokyo, Japan.
** Nippon Medical School, Tokyo, Japan.
*** Diponegoro University, Semarang, Indonesia.
**** Prince of Songkla University, Faculty of Veterinary Science, Thailand.
***** Khon Kaen University, Thailand.

genomic diversity in matrilocal and patrilocal communities. Nucleotide sequences for the EBV-*LMP1* were obtained from 53 mother-offspring pairs (residents of Sumba Island, Indonesia). The maternal contribution to the EBV transmission was less than half. An identical sequence shared by the mother and her offspring was found in 18 pairs (34.0%), whereas in 35 pairs (66.0%), different nucleotide sequences were identified between the mother and her offspring. A total of 189 sequences of the EBV *LMP1* gene were obtained for the ethnic residents in northern Thailand; 100 were from the patrilocal groups and 89 were from matrilocal groups. Nucleotide diversity (π) ranged 0.023 ~ 0.037 in patrilocal groups and 0.024 ~ 0.038 in matrilocal groups. EBV genomic diversity did not differ between the patrilocal and matrilocal groups ($p > 0.05$). These two approaches show that mothers do not necessarily contribute to the primary EBV infection in the earlier stage of life.

Introduction

Epstein-Barr virus (EBV), a member of the lymphocryptovirus (LCV) is a ubiquitous virus with high prevalence rates in human populations (reviewed in Henle & Henle, 1979). EBV is potentially involved in the development of several types of cell proliferative diseases including Burkitt's lymphoma, nasopharyngeal carcinoma, gastric cancer, salivary gland carcinoma, infectious mononucleosis, Hodgkin's disease and peripheral T-cell proliferative diseases (Epstein *et al*., 1964; zur Hausen *et al*., 1970; Burke *et al*., 1990; Saemundsen *et al*., 1982; Henle *et al*., 1968; Weiss *et al*., 1987; Mitarnun *et al*., 2002).

EBV is mainly transmitted via saliva (Yao *et al*., 1985); even healthy EBV seropositive carriers shed the virus into saliva but fetal infection has also been suggested (Goldberg *et al*., 1981; Meyohas e*t al*., 1996). The age of primary EBV infection varies geographically, possibly due to hygiene parameters. In industrialized countries primary infection occurs in adolescence with the symptoms in up to 50% of cases of tonsillitis, fever, malaise and lymphadenopathy, so-called infectious mononucleosis, (Niller *et al*., 2008). However, in underdeveloped countries, primary infection occurs in the early stage of life and is mostly asymptomatic. In Uganda, almost all residents are seropositive by three years of age (Meyohas *et al*., 1996). As for the EBV transmission route, the hypothesis that EBV is transmitted from mother to child has been proposed and up to now widely accepted (Miller *et al*., 1987; Evans *et al*., 1990; Minhas *et al*., 2010). Mothers spend much time taking care of their children and the practice of premastication may foster viral transmission to toddlers (Gaur *et al*., 2009). Trials to trace transmission routes of EBV by means of the EBV nuclear antigen (EBNA) size polymorphism failed because of the low resolution of size

difference (Gratama *et al.*, 1990). Therefore, the maternal contribution to EBV transmission remains unsolved. In the last couple of decades, EBV sub-strains have been defined by the sequence diversity in the latent membrane protein-1 (LMP-1) gene and the sub-strain distribution was also studied in the healthy populations (Saechan *et al.*, 2006, 2010). If EBV transmits from mothers to offspring, EBV genomic variability in a population should be influenced by marriage systems; that is, EBV genetic diversity would be greater in patrilocal groups than in matrilocal groups due to the movement of females with marriage.

Based on the anthropological field surveys, we employed two approaches to shed light on the EBV transmission mode, 1) direct comparison of the EBV genome between mother and infant, and 2) EBV genomic diversity in between matrilocal and patrilocal communities.

Survey 1

To evaluate the mother's contributions to the EBV transmission, as many EBV positive mother-infant pairs should were examined. It has long been demonstrated that there is a relationships between prevalence of EBV infection and socio-economic status. Lower socio-economic class shows earlier primary infection and higher prevalence of EBV (Henle & Henle, 1979). We therefore carried out a survey of subjects from low socio-economic societies still practicing a mostly traditional lifestyle.

Subjects and Method

A total of 347 healthy Sumbanese were recruited in East Sumba, East Nusa Tenggara Province, Indonesia (Fig. 1). After an informed consent was obtained from each individual or their guardian, venous blood samples were collected and whole blood DNAs were extracted.

An EBV DNA fragment (nt 168188-168618) corresponding to the C-terminal region of LMP1 (GenBank accession no. V01555.1) was amplified with the specific primers 5′-CCCCCACTCTGCTCTCAAA-3′ and 5′-CCGTGGGGGTCGTCAT-3′ (Saechan *et al.*, 2006) and a newly prepared primer set (5′-TGCTCGTGAGTGGAGCC-3′ and 5′-AGCTTAGCTGAACTGGGCC-3′). PCR conditions were set as initial denaturation at 94°C for 9 minutes followed by 40 cycles of at 94°C for 30 sec, at 60°C for 30 sec, at 72°C for 30 sec, and a final extension at 72°C for 10 min. PCR products were subsequently purified using a Microcon PCR kit (Millipore Corp.) and nucleotide sequences were determined using a BigDye™ terminator cycle sequencing Ready Reaction kit (Applied Biosystems). Sequencing was performed with a *3100-Avant*

Genetic analyzer (Applied Biosystems).

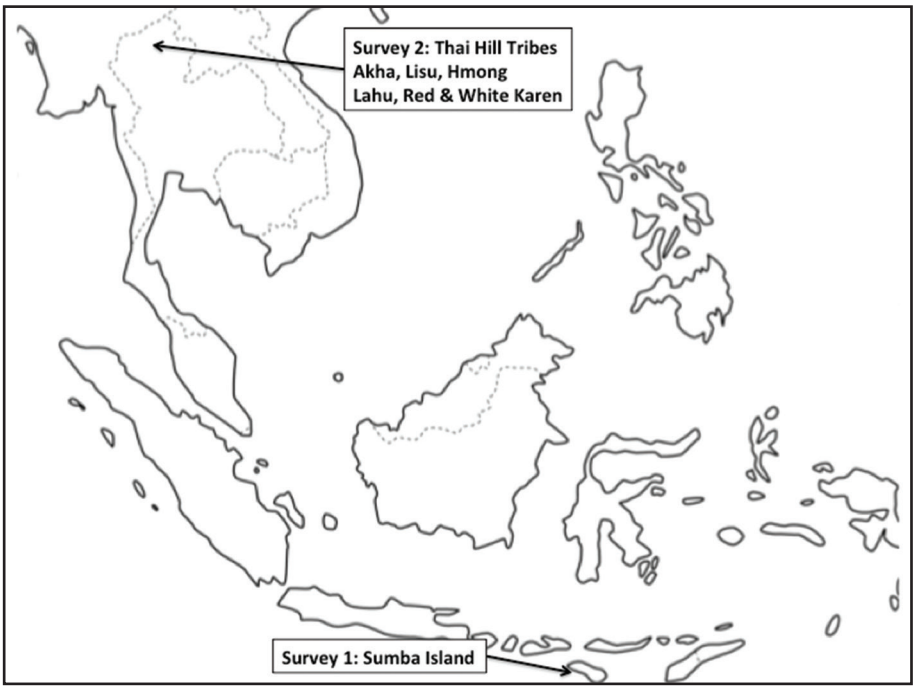

Fig. 1. *Location of the studied populations.*

Result and Discussion

Out of 347 DNA samples, 120 samples showed successful PCR amplification for the *LMP1* of EBV. Nucleotide sequences were obtained from 79 individuals (26 mothers and their 53 offspring) forming 53 mother-offspring pairs. In 18 pairs (34.0%), an identical sequence was shared by the mother and her offspring (=M group in Tab. 1), whereas in 35 pairs (66.0%), different nucleotide sequences were identified from the mother and her offspring (≠M group in Tab. 1). There were four offspring who carried an identical sequence to that of the father but not that of the mother. There was no difference in the maternal age at offspring birth between the =M group (23.44+ 5.33 yr) and the ≠M group (26.11+5.66 yr) ($p > 0.1$ by Welch's t test).

Transmission of EBV from mother to offspring via saliva seems to be an obvious hypothesis. However, this hypothesis was suggested and supported namely solely on the basis of epidemiological researches and the presence of EBV in carriers' saliva (Henle & Henle, 1979; Yao *et al*., 1985; Mbulaiteye *et al*., 2006). Our sequence analyses for the EBV

LMP1 clearly shows that the mother is not always the source of EBV in the offspring. Mothers accounted for about 34% of offspring EBV infections. EBV DNA concentrations suggested that salivary shedding of EBV in females was age dependent; it was higher in females <30 years (Mbulaiteya *et al.*, 2006). However, the mother's age at offspring birth did not alter the sequence agreement rate between mother and offspring (Tab. 1).

Category	No. offspring	Mother's age at offspring birth (yr)
=Mother	18	23.44±5.34
≠Mother	35	26.11±5.66
Total	53	25.21±5.69

Tab. 1. *EBV-LMP1 sequence in the offspring.*

Our present data are based on a single community in rural Indonesia where the people have maintained a traditional life-style such as wet nursing, and sharing bathroom with several families. These behaviors may promote EBV transmission from other individuals who are not the mother. This could account for the low rate of mother to offspring transmission. Although in this study we do not have data for all father offspring dyads, together our data indicates that the parental contribution to the EBV transmission to offspring accounted for more than 40%.

In high socio-economic communities, where a significant number of offspring may grow up naive to EBV, it is readily conceivable that mothers rarely contribute to the late primary EBV infection and therefore, a low agreement rate of shared EBV strain would be expected. The community in this study belonged to a so-called low socio-economic society in which EBV prevalence is high and the primary infection occurs in the early stage of life. In fact, 99% children under 10 years of age were seropositive for EBV infection (unpublished data); however, regardless of their age, a low agreement rate in EBV sequence between mother and offspring was demonstrated. We can thus presume an even lower prevalence of EBV transmission from

mother to offspring in higher socio-economic societies.

Survey 2

In order to confirm that the mothers were not the definitive source of EBV in the offspring, which was proposed in the Survey 1, we compared EBV genomic diversity in patrilocal and matrilocal ethnic groups.

A number of ethnic minorities are identified in Thailand (Chanbamrung *et al*., 1995); among them, Akha, Lisu, Hmong, Lahu, Red Karen and White Karen are known as the hill tribes living in northern part of Thailand. These populations are classified into patrilocal (females move out and males stay in their birth place) and matrilocal (males move out and females stay in their birth place) groups. Akha, Lisu, Hmong are regarded as patrilocal groups while Lahu, Red Karen, White Karen are matrilocal groups.

Subjects and Method

A total of 689 individuals (Akha: 199, Lisu: 123, Hmong: 98, Lahu: 54, Red Karen: 111, White Karen: 104) were recruited (Tab. 2). After obtaining informed consent, peripheral bloods were collected and then DNAs were extracted. The same EBV DNA fragment was analyzed by the same procedure stated in the section of Survey 1. Nucleotide sequences and nucleotide diversity (π) were analyzed in MEGA 5.0. Welch's method was employed for comparing π scores and P values less than 0.05 were considered to be significant.

Result and Discussion

A total of 189 sequences of the EBV *LMP1* gene were successfully identified with direct PCR sequencing; 100 were from the patrilocal groups and 89 were from matrilocal groups (Tab. 2). Nucleotide diversity (π) ranged 0.023 ~ 0.037 in patrilocal groups and 0.024 ~ 0.038 in matrilocal groups. EBV genomic diversity did not differ between the patrilocal and matrilocal groups ($p>0.05$).

If EBV is transmitted mainly from mother to offspring, EBV genomic diversity should be greater in patrilocal communities than in matrilocal communities because in the former communities females move between communities, which results in the large strain diversity of EBV. A comparable example is the haplotype diversity for maternally inherited mitochondrial DNA, which was higher in the patrilocal groups than in the matrilocal groups (Oota *et al*., 2001). Despite our initial expectation, there was no significant difference in the genomic

diversity of EBV in between the patrilocal and matrilocal groups; this contrasting result indicated that EBV transmission is not categorized as maternal transmission.

Group	No. Samples	No. of Sequence	Nucleotide diversity
Patrilocal	420	100	0.043±0.007
Akha	199	43	0.037±0.007
Lisu	123	26	0.030±0.006
Hmong	98	31	0.023±0.009
Matrilocal	269	89	0.040±0.007
Lahu	54	30	0.024±0.006
Red Karen	111	28	0.038±0.007
White Karen	104	31	0.035±0.007

Tab. 2. *Nucleotide diversity in each population.*

Conclusions

Survey 1 showed a low agreement rate of EBV-*LMP1* sequence between mother and offspring, while, Survey 2 showed EBV genomic diversity did not differ in matrilocal and patrilocal communities. It is thus concluded that the maternal contribution to the primary EBV infection in the earlier stage of life is not definitive and community life-styles may shape the EBV transmission route and positive rate.

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Corresponding author: Takafumi Ishida: tishida@bs.s.u-tokyo.ac.jp

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A milestone in the construction of the exhibits in National Museum of Ethnology, Japan: Japan World Exposition «Osaka 70 Expo» and its ethnographic collection

ATSUSHI NOBAYASHI*

Parole chiave: La Torre del Sole, missione etnologica Expo '70, collezione museale.

Riassunto — L'istituzione del Museo Nazionale di Etnologia, Giappone, deriva da una diversità di background. Questo articolo descrive l'influenza del materiale etnografico presentato nel 1970 all'esposizione mondiale «Osaka 70 Expo» nel padiglione dedicato a Minpaku, e si concentra sul ruolo di Umesao Tadao, primo direttore di Minpaku. Todao organizzò e contribuì alla raccolta di materiali per l'evento, ma non lavorò sull'allestimento. L'evento probabilmente incoraggiò Umesao a sviluppare un allestimento a Minpaku basato sulla ricerca etnografica e studi di teoria etnologica. Elementi importanti includono i metodi di raccolta dei materiali sul campo, così come la gestione delle informazioni sugli oggetti collezionati. In conclusione gli allestimenti attuali, che ci permettono di essere più vicini all'oggetto etnologico e di respirare la sua stessa aria, sono il patrimonio ereditato dall'esperienza di esporre a Expo '70 a Osaka e dall'attività di raccolta degli oggetti in tante zone diverse del mondo.

Key words: The tower of Sun, Expo '70 Ethnological Mission, museum collection.

Summary — The establishment of the National Museum of Ethnology, Japan comes from a variety of backgrounds. This paper describes the influence of the ethnographic material displayed in the theme pavilion of the Osaka World Exposition in 1970 on the Minpaku exhibit, focusing on the involvement of Tadao Umesao, the first director of the Minpaku. Umesao organized and contributed to the collection of materials for the exhibition but did not work on the exhibition. It probably encouraged Umesao to develop a Minpaku exhibit based on ethnographic research, ethnological theory and study. Important elements include the methods of the researcher going to the site to collect the materials as well as the management of the information on collecting objects. Finally, the exposed exhibition, which allows us to get as close as possible to the object and to breathe the same air as the object, are the kind of assets inherited from the experience of exhibiting at Expo '70 in Osaka and the activity for collecting materials from all over the world.

Introduction

The purpose of this publication is to provide a short introduction

* National Museum of Ethnology, Osaka, Japan.

to a certain collecting activity of ethnographic material that had a significant influence on the establishment of the National Museum of Ethnology, Osaka, Japan (thereafter Minpaku). The collecting activity was called «Expo '70 Ethnological Mission» (thereafter EEM). The EEM was formed to collect ethnographic materials from all over the world for the Japan World Exposition, Osaka 1970 (thereafter Osaka 70 Expo.) theme pavilion. The theme pavilion is «The tower of Sun».

Minpaku was founded in 1974 and opened in 1977 on the site of Expo '70 in Senri, Osaka. Although Minpaku is the name of the museum, its academic and administrative organization was set up as a research institute called the Inter-University Research Institute. The result is that Minpaku has different attributes from the other museums. The main purpose of Minpaku is to make available to the public the findings and results of research in ethnology, cultural anthropology, and related fields. Therefore, Minpaku has collected research materials in the humanities, especially, that pertinent to ethnographic research and cultural anthropology. The mission of Minpaku is accomplished by using these materials for research, the publication of its results, and the exhibits. What is collected in Minpaku's research activities is mainly items related to food, clothing, housing and other daily necessities of the people of the world. Minpaku's collection also includes religious items and artifacts for manufacturing.

Despite the fact that the Minpaku collection is an academic collection, Umesao Tadao, the first director of the museum, used an ambiguous word to describe the material when the Minpaku opened. It was «Garakuta». The Japanese word «Garakuta» generally means miscellaneous items and tools that are no longer useful. On the other hand, the term also contain positive nuances. The word Garakuta is sometimes used as guessworks of kanji, meaning «I have a lot of fun». Umesao's odd words that Minpaku's collection is Garakuta shows Minpaku's uniqueness. Through these words, Umesao seemed to hope that Minpaku would be a space where people would create new values of thoughts. The author thinks that one of the reasons why Umesao came to use this term is because of his experience of the Osaka 70 Expo. Strictly speaking, it was the activities of the EEM organized by Tadao Umesao.

The ethnographic materials collected by the EEM (thereafter EEM collection) were displayed in the basement of The tower of Sun during Osaka 70 Expo, and then transferred to Minpaku after they were administered by the Japan World Exposition Commemorative Association. The author organized a special exhibition on the EEM

collection in 2018 (Nobayashi, 2018). The purpose of the exhibition was to restore and reflect on the collecting activities of EEM (Fig. 1 and 2). In preparing for the exhibition, the author combed the literature documenting EEM activities and the exhibition at the Expo. Up until now, EEM records were written in Japanese by one of its representatives, Umesao Tadao (Umesao, 1973), and some objects of the collections were introduced in a few books in Japanese (Okamoto *et al.*, 1970, The Asahi Shimbun 1970). This paper introduces the context in which the EEM collecting activity took place and considers how it led to the exhibition at Minpaku.

Fig. 1. *Exhibition view of «Oceania» in the 40th Anniversary Special Exhibition of Minpaku «A «Tower of the Sun» Collection: Expo '70 Ethnological Mission» in 2018. (Photo by the author).*

Fig. 2. *Exhibition view of «Masks» in the 40th Anniversary Special Exhibition of Minpaku «A «Tower of the Sun» Collection: Expo '70 Ethnological Mission» in 2018. (Photo by the author).*

The predecessor museum of Minpaku

When we consider the background to the establishment of Minpaku, two important things need to be discussed. One was a request from the academic communities of Japan to establish an ethnological museum. The other was Osaka 70 Expo.

In fact, the present-day Minpaku had an ethnological museum predecessor. The previous ethnological museum was founded in 1938 in Hoya town, Tokyo (present-day Nishi-Tokyo city). The museum was managed by the Japanese Society of Ethnology and its gallery opened to public in 1939. The collection of the museum was donated to the Japanese Society of Ethnology by Sir Keizo Shibusawa (1896-1963).

Shibusawa was the grandson of Eichi Shibusawa (1840-1931), a prominent businessman known as the «father of Japanese capitalism». Keizo Shibusawa was himself an economist who served as Minister of Finance and Governor of the Bank of Japan. Besides being an economist he was also a researcher in ethnography.

Keizo Shibusawa was interested in the local cultures of Japan and especially engaged in ethnographic research on folk names of fish and fishing activities (Shibusawa 1993 [1936]). Shibusawa was also interested in material culture, especially tools for productive activity. Together with his friends, he collected tools and toys from all over Japan and established a private collection. To display the collected items, Shibusawa built an exhibition facility in part of his residence. Shibusawa created the «Attic *Museeum* Society», an organization dedicated to these studies, and the exhibition facility became known as the «Attic *Museeum*».

Later, Shibusawa donated his collection to the Japanese Society of Ethnology and provided financial support for the construction of a museum affiliated with the Society. The Museum of Ethnology, which opened in 1939, was closed for part of World War II. It reopened after the war, but the museum was then closed due to the age of the building and operational funding problems. The museum collections were taken over by the Ministry of Education in 1962 (Nakamura, 1984, 55).

The Japanese Society of Ethnology, the Anthropological Society of Nippon, the Japanese Archaeological Association, the Folklore Society of Japan, and the Japanese Association of Ethnology jointly submitted to the Minister of Education and other relevant authorities a request to establish a national ethnological research museum in 1964 (National Museum of Ethnology, 1984, 1-3). Bottom-up suggestions were also made by several academic organizations. Concreate plans for the new

museum, however, were never fleshed out at this point.

It was also a time when Japan was recovering from World War II. After 1950, overseas academic research resumed and anthropology and ethnography regained their vitality. The latter half of the 1950s, after Japan's international status was restored in 1952, saw a series of overseas academic surveys in the humanities and social sciences were planned. Initially, there were a number of systematic academic surveys that were conducted mainly by the University of Tokyo and Kyoto University. These surveys included Kyoto University Science Expedition at Karakoram and Hindukush in 1955 and 1956, the University of Tokyo's Iraq-Iran archaeological expedition in 1956 (Matsutani, 1997), the University of Tokyo's Andean Survey since 1958 (Onuki, 1997), and Kyoto University's African Ape Scientific Survey since 1961. Except for the expeditions organized by the University of Tokyo and Kyoto University, Equatorial Africa expedition by Waseda University, Patagonia expedition by Kobe University, Southeast Asia academic expedition by Osaka prefecture University, General survey on rice farming ethnic culture of Southeast Asia by the Japanese Association of Ethnology in 1957, Northwest Nepal expedition sponsored by the Japanese Association of Ethnology, Gorilla academic expedition by Japan monkey center in 1958 and Alaska academic expedition by Meiji university in 1960 (Iida, 2007, 245).

In 1962, the Ministry of Education's Grants-in-Aid for Scientific Research (commonly known as the «Kakenhi») established a category of «overseas academic research» and created an environment conducive to funding overseas field research. It was during this period in Japan that the importance of academic research with a global perspective was recognized in the humanities and social sciences, and this was supported by the insights of academic administration. The accumulation of these overseas research results also earned Japan credibility in the international academic community. In 1968 Japan successfully held the International Union of Anthropological and Ethnological Sciences (thereafter IUAES) World Congress in Japan.

Osaka 70 Expo and The World Expo. Task Force

While the academic community was working to build a new ethnological museum, plans for Osaka 70 Expo were underway. Osaka 70 Expo was held for 183 days from March 15 to September 13, 1970, in Senri Hills in Suita, Osaka, where the National Museum of Ethnology is now located. It was the first time in Japan that the International Bureau

of Expositions approved the holding of a World Exposition under the Convention on International Expositions. It was also a symbolic international project of post-war reconstruction following the 1965 Tokyo Olympics. The main theme of the expo was «Human Progress and Harmony», with the participation of 77 governments including Japan, 4 international organizations, 10 government agencies such as states and cities, 2 non-Japanese companies and 32 Japanese companies and industry associations. The total attendance of 642,18770, stood as a record until the 2010 Shanghai World Expo (Suzuki, 2018, 13).

The 1960s, when the idea for the Osaka 70 Expo was first conceived and the preparations for it were underway, it was a time of great change in the world. African independence began in 1960 and the Cuban crisis of 1962, saw an unprecedented arms race between the U.S. and the Soviet Union. In 1965, the bombing of the North in the Vietnam War began, and under the war without justice, the world began to search for a new order and value system. During this period, developed countries enjoyed the material wealth of mass production and mass consumption under high economic growth. At the same time, the need to consider the impact of rapid economic growth on the environment on a global scale began to be questioned. It was also a time of explosive population growth, limited food production, resource depletion, increasing, overt environmental pollution. In Japan, the anti-authority struggle, as represented by the school dispute, began to wane, and people's consciousness and social structure were steered towards prioritizing economic efficiency.

The idea of setting up a theme pavilion at the Osaka 70 Expo took shape in April 1966. The construction of a central facility was called for to concretely demonstrate the general theme of the Expo, «Human Progress and Harmony». In July of the following year, the artist Taro Okamoto was chosen as the producer of the theme exhibition. Okamoto, who worked on the theme pavilion, studied ethnology at the University of Paris when he was young. He originally belonged to the philosophy department but moved to the ethnology department after being attracted to the Musée de l'Homme, built on the site of the 1937 Paris Expo. Okamoto later recalled that his university lectures were held at the museum, surrounded by exhibitions and vast collections of artifacts, and immersed in a dreamlike space where he immersed himself in the lectures of famous professors such as Marcel Mauss and Paul Reve (Umesao, 1978, 29). This experience seems to have imbued Okamoto's thinking with the idea of building a museum after the Expo.

Okamoto conceived the structure of the thematic exhibition as

underground-past, above-ground-present, and aerial-future, and his aim was for these three spaces to resonate with each other while remaining independent, and to exist as a cohesive whole. And Okamoto designed the Tower of the Sun as a space to make this space a reality (Fig. 3). Okamoto believed that the underground space represents the root of human life that sustains the ground and the air. Okamoto asked Sakyo Komatsu (1931-2011) to be a sub-producer to consider the contents of this underground exhibition.

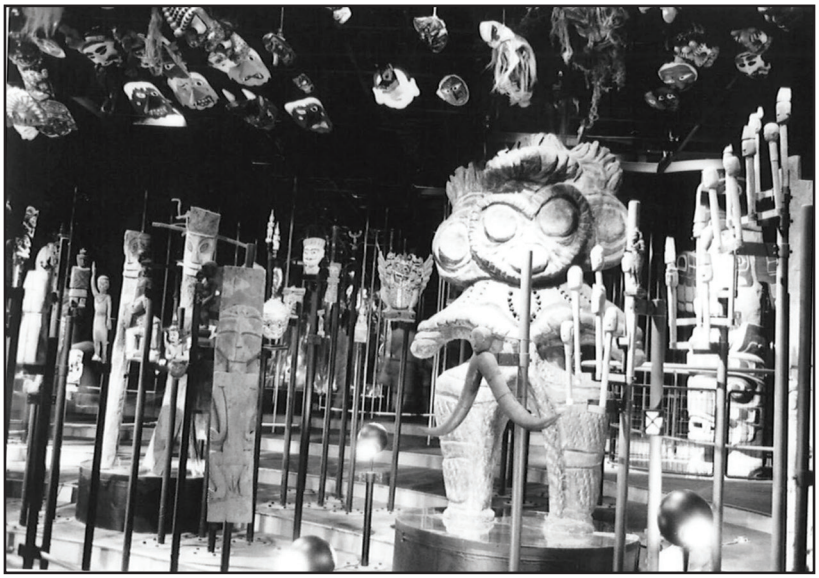

Fig. 3. *The exhibition of Masks and Statues of gods in the Tower of the Sun at the Osaka 70 Expo (Courtesy by Institute of Esthetic Research).*

Komatsu was an up-and-coming science fiction writer at the time. After graduating from Kyoto University, he did not have a regular job, but made a living by working part-time and writing. He began writing science fiction novels that appeared in newspapers around 1960. In 1962 he made his full-scale debut in a commercial magazine under the writer's name Sakyo Komatsu (2019, 4328-4329/4544). At that time, Komatsu was in charge of a series of articles in the monthly newspaper «Housou Asahi (Broadcast Asahi)», published by Osaka Asahi Broadcasting. It included not only critiques on broadcasting and mass media, but also discussions and essays on informative culture and civilization from a broad perspective. In fact, in 1963 Komatsu got acquainted with Tadao Umesao through his work at the magazine (Komatsu, 2019, 2717/4544). In 1964, Komatsu and Umesao, together with several other colleagues,

organized a private study group, «Bankokuhaku wo kangaeru kai» (The World Expo Task Force). They began to discuss the historical background of the birth of the World Expo, the form of the past Expo, and the significance of holding the world Expo in Japan (Komatsu, 2019, 2911-2717/4544). The Preparatory Committee for the International Exposition asked this private group for their opinions on the World Expo, and Komatsu and Umesao became involved in the Expo project.

«Expo '70 Ethnological Mission»

Before Okamoto was chosen as producer, Umesao and Komatsu drew up the following drafts for the sub-themes of the Expo; (1) Man (Life) Itself, (2) Man and Nature, (3) Man and Technology, (4) Man and Man, and corresponding to (1) more abundant life, (2) more abundant use of nature, (3) more desirable life design, (4) deeper mutual understanding. (Komatsu, 2019, 3489/4544). It is not yet clear to what extent Okamoto incorporated these aims into the design, but the completed underground exhibition space in the Tower of Sun was 44 meters long with a section on «Life», from the birth of life from materials on earth to the evolution of life, 50 meters long with a section on «People», where human history unfolds, and 100 meters long with a section on «Heart», where masks and statues from all over the world were displayed, ranging from tools of daily life to masks and statues. Okamoto left the following description about this exhibition.

«I want to put there the compelling and proud evidence of human culture. I wanted to bring to light the limpid realization of how human beings have come to appreciate life since they became «human». They once led to the whole world, but they have been destroyed by modern civilization and are now disappearing. In order to take advantage of the unique opportunity of the upcoming World Expo, I poured all my passion into planning for it from all over the world» (Okamoto *et al.*, 1970, 2).

Okamoto's words are quite rightly regarded as a social evolutionist idea that pays no attention to the cultural context of the local community. In fact, there is no record of Okamoto's logical explanation of what kind of exhibits are needed for this purpose. The author thinks that Okamoto wanted to show «what humans are and where they come from» through the exhibition of the basement of the theme pavilion. This is because Okamoto's experience of studying anthropology at the Musee de L'Homme during his young days has undeniably had an influence on Okamoto.

Okamoto also wanted visitors to feel a sense of the reality of the object, so he planned an exposed exhibition without using any cases. In other words, the goal was to create a space where things and people exist in the same air. Adopting this method of exhibition meant that they had to abandon the idea of borrowing materials from existing museums. Plans were made to collect materials from around the world to be used in the exhibit. Two anthropologists, Seiichi Izumi of the University of Tokyo and Tadao Umesao of Kyoto University, were commissioned to carry out this collection project.

Izumi and Umesao had been planning the collection plan for the exhibition in the basement of the Tower of the Sun since May 1968. They divided the world into Japan, Korea, Taiwan, Southeast Asia, India and the Middle East, East Africa, West Africa, Europe, North America, the Latin American Highlands, the Latin American Amazon, and Oceania, and asked young researchers with local knowledge of each region to go on collection trips. The fact that continental China and the Soviet Union were not included in the collection area is a true indication of the diplomatic situation at the time.

Izumi and Umesao were also members of the museum promotion committee of the Ethnological Society of Japan at that time. However, the collection of ethnographic materials for the Osaka 70 Expo and the construction of an ethnographic museum were two separate issues at the time. Okamoto also played an important role in the opportunity to tie these two issues together.

In the summer of 1968, the IUAES was held in Tokyo and Kyoto, Japan. On September 6, a party was organized by the Japan Association for World Expositions to hear from researchers from various countries about the possibilities and methods of collecting ethnic materials. A prominent anthropologist was invited to the party (Tab. 1), and the sender of the invitation was Taro Okamoto (Umesao, 1973, 18-19, Fig. 4.). Of course, Izumi and Umesao were also present at the party. A handout there stated the types of materials the EEM planned to collect and that an ethnographic museum would be built within a few years after the Expo and the materials would be housed there. Umesao interpreted this as explaining why the researchers who participated in the IUAES were invited to a meeting organized by the Japanese Association of World Expositions (Umesao, 1971, 20). As a result, this meeting had a significant impact on the EEM's collection activities, as in some cases collection activities relied on the attendees of this meeting and the method of exchange of museum materials proposed at the meeting.

Name	Affiliation
Shunichi Suzuki	Secretary General of the Association of Japan World Exposition
Yuji Hirai	Deputy Secretary General
Tomoo Hirose	Chief of Theme Section
Taro Okamoto	Theme Exhibition Director
Sakyo Komatsu	Vice-Director of Theme Exhibition
Shigeomi Hirano	General Manager of Institute of Esthetic Research
Seiichi Izumi	Professor of Institute of Oriental Culture, University of Yokyo
Tadao Umesao	Associate Professor of Research Institute for Humanistic Studies, Kyoto University
Junzo Kawada	Associate Professor of Saitama University
Tadahiko Hara	Research Associate of Institute for Asian and African Languages and Culture, Tokyo University of Foreign Studies
Hiroko Hara	Associate Professor, Takushoku University
Tamotsu Aoki	Research Associate, Institute of Oriental Culture, University of Tokyo
Naomichi Ishige	Research Associate, Research Institute for Humanistic Studies, Kyoto University
Berndt, Ronald M.	Professor, University of Western Australia, Commonwealth of Australia
Reichel-Dolmatoff	Professor of Los Andes University, Republic of Colombia
Riviere, Georges	Ancient Directeur du Musée d'Arts et Traditions Populaires, French Republic
Lee, Duhyun	Professor, Seoul National University, Republic of Korea
Agblemagnon, Ferdinand	Director of National Institute of Sociology, Republic of Togo
Bromley, Yu V.	Director of Institute of Ethnography, Academy of Science, Union of Soviet Socialist Republics
Chard, Chester S.	Professor of University of Wisconsin, United States of America
Gunther, Erna	Professor of University of Alaska, United States of America

Tab. 1. *Attendants of the meeting.*

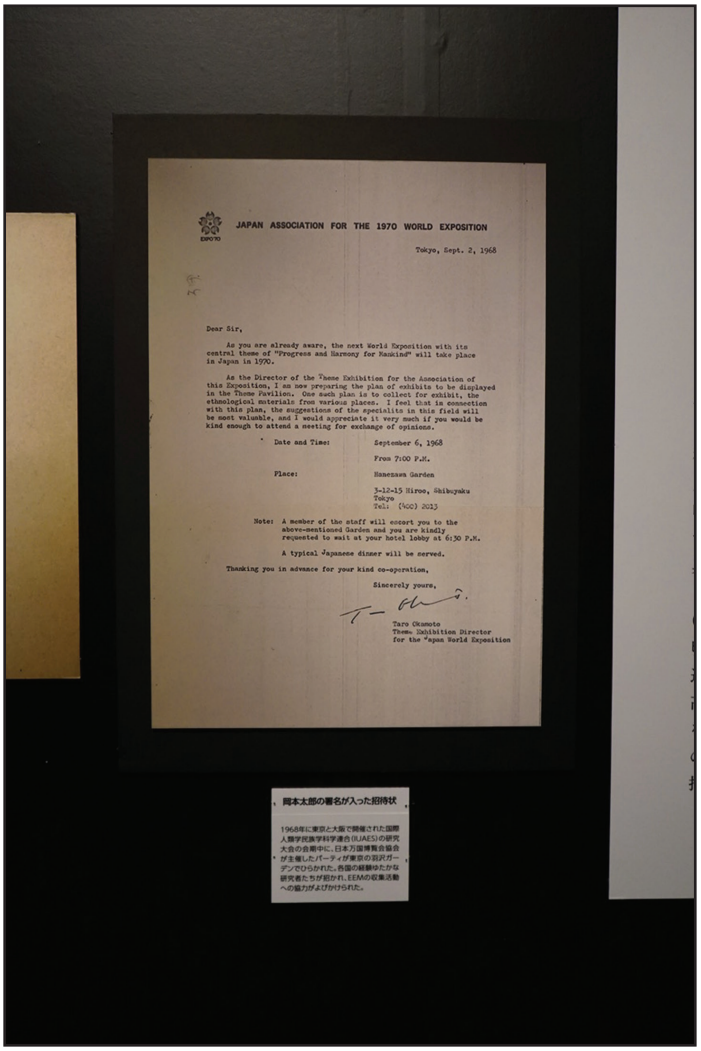

Fig. 4. *Invitations to researchers to attend the meeting (Umesao Archives No. Ume_EEM_1_028_001_001_f copy, photo by the author).*

Those involved in the Osaka Expo came to see the EEM materials and the Ethnological Museum as a combined matter, while the academic community did not necessarily share the same view. Umesao gives several reasons why he was unable to bring the idea of combining EEM's activities with museum construction to the academic community.

One is that, cooperating with the Expo to carry out the collecting project did not guarantee that a museum would be built. Umesao and Izumi were well aware that the construction of the museum was a state

project and had no direct relationship to the project of the Osaka 70 Expo. The second reason was that the budget for EEM collection was too small. Although the cost of purchasing and transporting the materials was barely sufficient and the cost of transportation and lodging for the collectors had to be severely budgeted. There was not enough money to pay the salaries of collectors and they had to work on a volunteer basis. For this reason, Izumi and Umesao could only ask their closest researchers to help them. The third reason was the circumstances of the time in which the collection was planned.

During this period, conflicts arising from the student movement were very common at universities across Japan. The University of Tokyo, where Izumi taught, cancelled the entrance examination in March 1969 due to a school dispute. Some scholars in the academy objected to the holding of the World Expo and criticized the collection of ethnographic material from all over the world as an act of colonialist plunder. Some researchers Izumi commissioned resigned. As a result, it was decided that Umesao would be responsible for selecting the members who would engage in collecting the materials of the vacant areas. Later on, these problems seem to have had no small influence on the organization of the National Museum of Ethnology. Of the 15 members who were essentially tasked with collecting overseas materials and serving as the secretariat, seven were later hired as museum members at the start of Minpaku.

EEM collection and the exhibition in the theme pavilion

Eighteen people were eventually involved in collecting the materials. They had an average age of about 30 years and included university teachers, graduate students, newspaper reporters and engineers. The total budget for the project was 6,000,000 Japanese yen, which included not only the purchase of the materials, but also the cost of transportation, travel expenses and accommodation for the collectors.

The author will leave a detailed description of the collection activities of each EEM member for a future paper and just show the number of objects of collection and its original place according to the record (Umesao, 1973; Nobayashi, 2018). The number of objects was 2,497 from 51 countries and areas. Its breakdown is as follows; Japan; 266, Korea; 133, Taiwan; 119, Indonesia; 58, Cambodia; 21, Thailand; 105, Burma; 6, Philippines; 30, Vietnam; 1, Borneo; 1, Malaysia; 48, Afghanistan; 51, Iran; 59, India; 196, Ceylon; 4, Nepal; 30, Pakistan; 51, Lebanon; 12, Uganda; 65, Ethiopia; 15, Somalia; 2, Tanzania; 60, Madagascar; 7,

Malawi; 4, Africa (anonymous); 27, Upper Volta; 1, Ghana; 73, Cameroon; 19, Ivory coast; 33, Central Africa; 23, Nigeria; 2, Mali; 3, Italy; 58, Spain; 1, Hungry; 17, Bulgaria; 56, Yugoslavia; 81, Romania; 94, Guatemala; 2, Columbia; 147, Mexico; 94, U.S.A.; 124, Australia; 30, Samoa; 13, Tahiti; 6, New Guinea; 6, New Hebrides; 12, Micronesia; 18.

Since the purpose of the collection was to collect masks and statues of gods from different parts of the world, the proportion of masks and statues of gods in the collection was understandably high. Some of the statues of the gods and masks had already been produced as Souvenirs. Meanwhile, a variety of materials were collected by the EEM, including musical instruments, artefacts, furniture and clothing.

The collected materials were displayed in the basement of the Tower of the Sun, the theme Pavilion of Osaka 70 Expo. It was Okamoto Taro and sub-producer Komatsu Sakyo who was responsible for the exhibition and no members of the EEM, including Izumi and Umesao, were involved in the exhibition. Umesao was an invited guest at the opening ceremony of the Expo and had the opportunity to see the basement exhibition of the Tower of the Sun. Umesao said the following about the impressions he received from the exhibit at that time.

«An exhibition at the World Expo is not an academic exhibition in itself, as it is done from a unique standpoint in accordance with the theme and under the technical conditions of the exhibition. However, the materials themselves can be used as materials for academic exhibitions in museums if they are displayed in a different way» (Umesao, 1970,10).

In a sense, we can think of Umesao as expressing his disappointment with the exhibition at the Expo. Umesao continued to read the letters from EEM members who had been dispatched to various parts of the world, describing their experiences in the field. Through the eyes and writings of the EEM members, Umesao was able to see the social conditions in which the collected materials existed and the people who made and used them. For Umesao, the exhibition that did not refer to any of the various cultural and social situations related to the objects at the Expo must have been something that he could not evaluate as academic. It is not surprising that Umesao had the desire to create an academic exhibition at the museum using the same materials as the Expo.

«Garakuta» in the solitary amusement: crux of the matter in the Minpaku

The first time Umesao used the word Garakuta to describe the Minpaku materials was in a conversation with Sakyo Komatsu. The contents of this interview were published in the first issue of Monthly Minpaku, a public relations magazine that was published before the museum opened. Komatsu was chosen as Umesao's first interlocutor.

At the beginning of the interview, Komatsu asked Umesao how the idea of establishing the Minpaku was born. Komatsu asked Umesao if he had a plan to make the museum unique and different from the existing Japanese museums. Umesao responded to this question as follows;

«The plan to create an ethnological museum itself is more than 40 years old. In the beginning of the project, it was called the Museum of Ethnological Research. The plan was developed in response to a very strong request from researchers. And so, consistently from the beginning, the Minpaku was a research institute in nature. [...] We conduct ethnographic research and to collect, store, and exhibit ethnographic materials for public viewing. [...] Legally, Minpaku was established as one of the national inter-university research institute. Seven national inter-university research institutes have been built to date, but they are research facilities within the framework of a broadly defined national university. Most people think that museums are under the jurisdiction of the Agency for Cultural Affairs or the Social Education Bureau of the Ministry of Education, but Minpaku is under the jurisdiction of the Science and International Affairs Bureau of the Ministry of Education.

The materials collected and displayed by Minpaku are also different in nature from those in other museums. People generally think of a museum as an institution for the protection of cultural properties. The three national museums in Tokyo, Kyoto and Nara are all affiliated with the Agency for Cultural Affairs and have a clear legal commitment to the protection of cultural properties. However, the materials collected and exhibited at the National Museum of Ethnology are not cultural properties. If it wasn't a cultural property, I wouldn't want to be asked what it was, but I'd have to answer, Well, it's 'Garakuta'. In fact, to be precise, it's an academic research resource. That is the nature of what you will see when you come to the Ethnology Museum. It's not a treasure by any means» (translated by the author, Umesao, 1978, 6).

After some of exchanges with Komatsu, Umesao said the museum

collection policy and function as follows;

«If they are left, they will be burned to the ground by people. We collect them before they are burned. This is our purpose. We shall collect those things that are most closely related to the daily lives of ordinary people» (translated by the author, Umesao, 1978, 7).

Umesao also pointed out the important difference between exhibitions in Expo exhibition and a museum.

«Of course, there are differences between an exposition and a museum in the way they are displayed. The differences I have in mind are as following. For example, there are many different plants. We are going to collect a flower for each one and make them into a magnificent flower arrangement. The audience is astonished. It is an exposition. On the other hand, we break up the flowers into individuals, and grouped the same plants into a botanical system, or arranged by region, so that the plants of the world and their distribution can be better understood. Or arrange them in such a way that the evolution and phylogeny of plants can be better understood. It is a museum exhibit» (translated by the author, Umesao, 1978, 14).

Umesao also said that the exposition was characterized by «astonishment» and that it was a festival of civilization that required a certain kind of excitement, and on the other hand, the museum was not a festival for people to enjoy together but a solitary amusement in which people were confronted with the exhibits and themselves (Umesao, 1978, 16).

The structural arrangement of the materials described by Umesao was called «a structural exhibition» and was the basic concept of the first Minpaku exhibition. This holistic view that tries to capture the whole system and structure of culture may have originated from Umesao's background as a specialist in natural science, especially in ecology. Cultural diversity is a popular term, but Umesao's perspective was focused on the variations in civilization (Umesao, 1998 [1957]). Umesao was wondering if he could explain the variations in civilization that human populations, as a group of the same species, have created through their historical activities by using the biological approach of ecology.

These ideas of Umesao were reflected not only in the specific methods of exhibitions, but also in the methods of organizing the facilities and collections of the Minpaku. At the time of its founding, Minpaku signed a contract with Yale University and became an official member of the HRAF. HRAF is the common name for the Human Relations Area Files, an inter-university organization started in 1949 with a grant from the

Carnegie Foundation. The headquarters are located at Yale University in the United States. The Minpaku has adopted the OCM (Outline of Cultural Materials) and OWC (Outline of World cultures), classification systems for cultural subjects and ethnic populations developed by the HRAF project, for its collections and library materials. It would be an insightful indiscretion to encode the cultural context of a material culture that is somewhat subject to descriptive research. Especially in today's world where the IT environment for big data analysis is easily accessible, the structural organization of the materials that Umesao introduced can be considered as a great legacy of Minpaku.

Summary and Conclusions

Nearly half a century has passed since the symbolic term «the museum as a forum» was first introduced to the world. Duncan Cameron, a museum researcher, published a paper aimed at the museum world in 1971 with the provocative title «The Museum: temple or forum?» (Duncan, 1971). Since then, this phrase still seems to have a strong influence on people involved in museum studies and museums themselves. However, it cannot be denied that the repeated translations and interpretations of this paper have led to a tendency to emphasize a forum feature. In fact, Duncan himself has not stated that he wants to turn museums into social clubs or funfairs. While acknowledging the museum as a temple, he said it needs to be reformed to make it better and more effective (Duncan, 1971, 17).

The forum can be thought of as basically a space for people to come together. It is interesting to note that Umesao, who envisioned the construction of a new museum at the same time, described the realized museum as an amusement of solitude. It can be understood as Umesao's criticism of the museum as a product of civilization. It is a question if the museum can be seen a space for people to come together and feel empathy as a group is really a justified entity. The author thinks that Umesao placed great importance on an individual's intellectual curiosity and the ability to think. It was expressed as the term amusement of solitude. Umesao came up with this idea because he experienced a big gap between the collection of EEM and the exhibition using the collected materials for the Osaka 70 Expo. Umesao may have felt that the large number of visitors to the exhibition did not gain anything by looking at the ethnographic materials, which were arranged without any explanation or theoretical rules, as if the visitors were just going through the motions.

The exhibition at the Theme Pavilion at the Osaka Expo fostered Umesao's ideas about the methods of displaying ethnographic materials. Umesao's impression of the exhibition in Osaka 70 Expo, which can be seen as a disappointment, suggests that Umesao felt that ethnographic materials could be a resource for intellectual production or useless «Garakuta», depending on how they are treated. While Umesao thought that museum exhibits should be viewed by individuals, the materials should be viewed collectively according to certain rules. It might be in stark contrast to an art museum, where a large number of visitors view a single high-value piece of art. For Umesao, the museum was an ecological environment where things were interconnected with each other, including the exhibition space. Ethnographic material, which is a single «piece of junk», becomes useful as an ecological environment for understanding different cultures when it is reconstructed according to some explainable theory.

Corresponding author: nova@minpaku.ac.jp

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Scientific Voyages towards Japan through the collections of the Museum of Anthropology and Ethnology of Florence

FRANCESCA BIGONI* MARIA GLORIA ROSELLI*

Parole chiave: collezionismo, orientalismo, Ainu.

Riassunto — In questo studio offriamo una visione dell'attrazione verso il Giappone che è sempre stata presente fin dalle origini del Museo di Antropologia e Etnologia di Firenze e che si è materializzata in racconti di viaggio, immagini fotografiche, collezionismo ed esposizione di artefatti. Nelle diverse epoche storiche attraversate dal Museo fino a tempi recenti, vetrine con oggetti giapponesi sono sempre state incluse nel percorso di visita destinato ai visitatori. Sottolineiamo l'importanza particolare della collezione di artefatti Ainu arrivata in museo grazie all'etnologo e fotografo fiorentino Fosco Maraini.

Key words: collectionism, orientalism, Ainu.

Summary — In this study we offer a vision of the attraction towards Japan that has always been present since the origins of the Museum of Anthropology and Ethnology in Florence and which has materialized in travel stories, photographic images, collecting and exhibiting artifacts. In the different historical periods crossed by the Museum until recent times, showcases with Japanese objects have always been included in the visit path intended for visitors. We underline the particular importance of the collection of Ainu artifacts that arrived in the museum thanks to the Florentine ethnologist and photographer Fosco Maraini.

Introduction

In 1869, when Florence was capital of Italy, Paolo Mantegazza founded the National Museum of Anthropology and Ethnology. In the following few years and tightly linked to the museum, he also founded the Italian Society for Anthropology and Ethnology (Società Italiana di Antropologia e Etnologia) and a scientific journal, the Archive for Anthropology and Ethnology (Archivio per L'Antropologia e la Etnologia). Paolo Mantegazza was the first Italian anthropologist: he held the first Italian chair of Anthropology in the «Istituto di studi superiori» (Institute of superior studies), today known as the «University of Florence». He was recognized as a multifaceted talent, an influential

* Natural History Museum, Anthropology and Ethnology section, University of Florence, Via del Proconsolo 12, 50122, Firenze, Italy.

intellectual across Europe. His career included many areas of expertise and activities: medical pathologist and hygienist, traveller, professor, patriot, senator of the young Italian Nation, Founder and President of the Italian Photographic Society (Fig.1). Mantegazza's stature is shown by the exchange of correspondence with Charles Darwin, the most famous scientist of the era, who also cited his scientific publications (Bigoni and De France, 2014). Modern anthropology was founded in Italy by Mantegazza on the firm ground of the theory of Evolution. The Museum of Anthropology and Ethnology and the Italian Society of Anthropology and Ethnology with the Journal «Archivio» were linked to a broad international context.

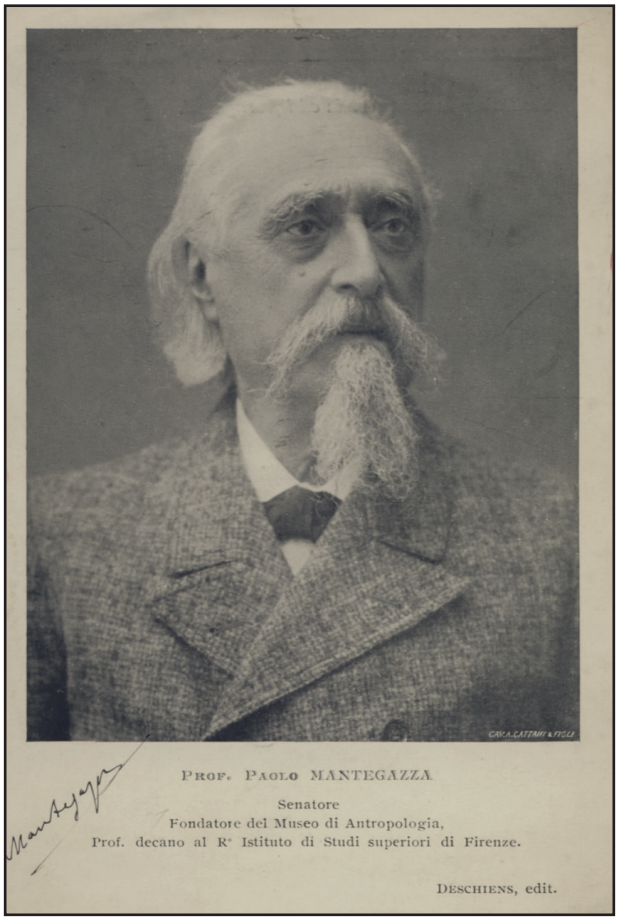

Fig. 1. *Paolo Mantegazza.*

Enrico Hillyer Giglioli and Japan

One of Mantegazza's closest collaborators, active in the Society, was Enrico Giglioli. He is mostly known and cited as a zoologist, because at the time he taught Comparative Anatomy in Florence. But he was also very influential in the development of the new ethno-anthropological disciplines, especially in the museological field. The careers and lives of Enrico Hillyer Giglioli and Paolo Mantegazza have many common aspects. Both held roles as Vice President and President, respectively, of the Italian Society of Anthropology and Ethnology. In addition, both were professors in the same 40 years at the Institute of Advanced Studies, and directed Museums in their respective areas of expertise, which are now united in the Natural History Museum of the University of Florence. Both began their careers making voyages to distant continents, voyages that left an indelible mark on their cultural formation and scientific interests. The material and cultural exchanges between the two men were multiple and they formed a friendship that began when both arrived in Florence in 1869 and lasted for the rest of their lives (Barbagli, 2014). In his youth, Mantegazza had spent a long time in South America, later he travelled to Norway (Lapland) and India. These trips and contacts with the populations of these regions greatly influenced his career and left an important trace in the collections preserved in the Museum.

Giglioli had a different experience. After studying in England where he was a student of Thomas H. Huxley, he participated in an important circumnavigation trip on the ship «Magenta», which also took him to Japan. Giglioli's role during the voyage concerned naturalistic study and collection of samples, as Darwin had done on the Beagle, but he also ended up as a translator and had the opportunity to participate in the whole process that led to the signature of the historic First Treaty of Friendship and Commerce stipulated between the newborn Italian State (1861) and Japan on August 25, 1866 (Fig.2).

In his book Giglioli made a detailed description of places in Japan: he was especially captured by the beauty of Kamakura. Further, he took also this opportunity to write about the history of Japan, the political system and the spiritual life of his inhabitants. Interestingly, even if Giglioli did not have the chance to visit the Ainu villages in Hokkaido, he collected all sorts of information and wrote about Ainu in his book. His experience in Japan was very important in Giglioli's human and professional development: he dealt extensively with this topic in the account of the voyage aboard the Magenta (Giglioli, 1875), and he often

returned to it in articles written in the following years. The intense activity of Giglioli, so tightly connected to the Society of Anthropology and Ethnology and to the Museum of Florence, certainly played an important role in defining an interest for the Far East particularly for Japan.

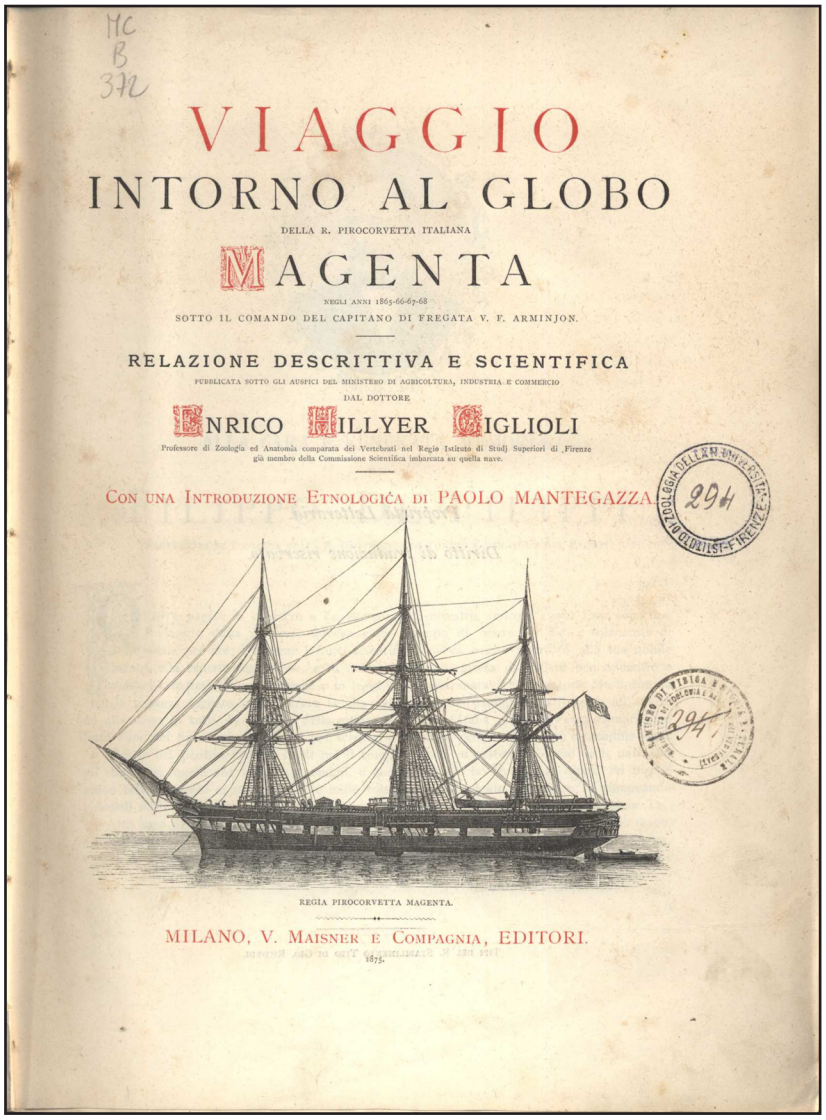

Fig. 2. *Giglioli's book about the journey that took him to Japan.*

Collections in the museum

Giglioli's emblematic experience reflected a strong European interest in Eastern cultures and especially in Japan. The attraction for objects from the Far East by Europeans is illustrated by a long history of collecting, artistic contamination, cultural dialogue. The Museum of Anthropology and Ethnology houses a heritage from many different cultures and eras, and also is an expression of this interest for the far East.

It is notable that Museum of Anthropology was founded during a time of great vitality in Oriental studies. This pervaded not only the academia, but also the whole world of arts and culture, always looking for new ideas and inspirations (Roselli, 2014, 77). These multiple fields of study are reflected in the varied origin of the Japanese collection, collected and received in the museum by scholars of various disciplines, artists and individuals who found themselves traveling or spending long periods in the Far East on business.

One of them was an illustrious member of Florentine culture in the late 19th century, Arnold Henry Savage Landor (1865-1924). Grandson of the English writer Walter Savage Landor, he was born and raised in Florence, showing an early inclination for figurative art. He travelled a great deal and supported himself by painting portraits of important and rich people in the countries he visited. A collection of 200 paintings, sketches, drawings and watercolours conserved in the museum portrays the peoples and landscapes of Japan, Korea, China, the Philippines and Tibet. The artist's gaze was turned to exotic scenes, but he always employed Western techniques and sensibilities. Another 100 watercolours depict the Ainu of Hokkaido with great vividness and immediacy, as if they were snapshots of everyday life. He visited this people in 1889, «alone and without friends, servants or guides, with minimal baggage, without provisions or tent», as he was careful to specify in the account of his journey *Alone with the Hairy Ainu* published in 1893. He also brought a collection of objects back from his travels, including some *Iku-bashui*, the ritual sticks from the Ainu culture. The collection was inherited by the Maganzi family, which had bonds of friendship and kinship with Landor, and which donated the entire collection to the Museum of Anthropology in 1955.

More *iku-bashui*, together with other objects and a samurai warrior attire, came from a nineteenth-century collections donated by the Faculty of Letters and Philosophy of the Florentine University. In fact, within the Florentine academy, from 1860 until the end of the century,

studies and research flourished around the oriental disciplines. Speacialized academic chairs were established and classes held which projected Florence onto the international scene for their high level of quality. Thanks to the efforts of a large and cultured group of orientalists, the University also managed to acquire the Medici Printing House, founded in the 16th century in Rome for printing in foreign languages. Using and expanding the range of punches, they managed to print volumes directly in the Japanese language.

Japanese paintings, pottery and small terracotta sculptures are included in the collection of Erasmus Ehrenfreund dated 1901-02. He was a scholar very active in the «Società» who, among other things, put together the huge bibliography of Paolo Mantegazza's works after his death. Beautiful artifacts were collected by the Florentine ethnologist Lamberto Loria at the end of the 19th century.

Giovanni Branchi was another donor of an Asian collection in the Anthropology section. He was a non «scientific» traveller who journeyed and lived in many countries as an Italian diplomat. His eastern destinations included Japan, China and India. The Japanese collection, consisting of over 100 items, includes numerous *netsuke, inro, katana* swords and even a full armour. His very refined and beautiful collection is an example of the nineteenth century «exotic» taste towards the east expressed by wealthy and culturally prepared travelers. In fact, Branchi collected the objects for his private collection, which he decided to donate to the Museum once his long career as a diplomat around the world was over.

Recently the museum received a donation by the Japanese master Katsumi Oda. It includes twenty-four Noh theatre masks, made of cypress wood and painted with lacquer.

The Maraini Collection

Fosco Maraini (Florence 1912 - Florence 2004) just before outbreak of WWII left Italy for Japan. In 1938 he arrived in Hokkaido thanks to a scholarship that took him to the University of Sapporo with the purpose to study the Ainu people.

«I had the fortune in my first encounter with Ainu culture the help and precious suggestions of Neil Gordon Munro, a Scottish medical doctor. Munro lived with the Ainu of Nibutani village and provided them with free medial treatment. He was held in high regard by the Ainu. My residence was in Sapporo and periodically I moved along the east coast of Hokkaido where there were still tens of Ainu villages. There I made my research. On a couple of occasions I traveled as far as to visit

some villages along the coast of Sachalin. At that time the southern part was under the Japanese and was called Karafuto» (translated from Maraini, 2001, 173).

During his research he collected more than 450 objects. The *Iku-bashui* held a particular interest for Maraini and he published a detailed study about them in 1942. Maraini had the possibility to observe elders, the *Ekashi*, using these special sticks to send ritual offerings to the gods: *I thought that the systematic study of the numerous signs that ornamented isku-bashui might be a usefule key to better understand their ideological and religious system. This research was greatly facilitated by the elders of Nibutani who had a perfect knowledge of the hundreds of different symbols. They were very eager informatants* (translated from Maraini, 2001, 175).

All the collected artifacts, included the series of precious *Iku-bashui*, were put in 51 cases and miraculously survived the war, preserved for years in the basement of the French Institute in Kyoto. In 1948, after Maraini was able to go back to Italy, the collection was donated to the Museum in Florence. In the following years Maraini added also a documentation of photographs and film.

Maraini maintained for his entire life a great passion for Japan. His books full of photographs introduced Japan to many westerners. We can cite his book «Ore Giapponesi» (Japanese Hours) first published in 1957 that introduced Italians to the «mysterious» and fascinating Japanese culture. This book was later translated into German, Spanish, French and outside Europe in English with the American edition. The American edition, with the title «Meeting with Japan», become a best seller: it was a Book of the Month selection and was printed in 200,000 copies. Maraini modestly wrote, «Probably it was luckily published just when people wanted to know more about Japan» (2001, 184).

Japanese artifacts in the Museum today

Today the Florentine museum has a hall dedicated to the Ainu collection of Maraini. It is an extraordinary testimony of the distinct culture coming from Hokkaido. In the introductory hall of the museum there is a octagonal case with examples of a small number of Japanese objects including *netsuke* and *inro* (Fig. 3-4). This small exhibit originated from 2015, when the museum became the venue to celebrate the 50 anniversary of the twin cities of Kyoto and Firenze. The twin cities pack between these two great cities of art dates to the 6 November 1965 signing in Florence by the major of Kyoto, Soichi Nogami (professor of Italian language, literature and famous Dante scholar) and the major of Florence Lelio Lagorio. Fifty years later, for the renewal of the twin

agreement, the museum hosted a cerimony and reception with the participation of the majors of both cities, Daisaku Kadokawa and Dario Nardella as well as the Japanese ambassador, Kazuyoshi Umemoto. The public was impressed by the delicate beauty of the accompanying *Maiko* from Kyoto.

Fig. 3. *Netsuke from the Florentine collection.*

Fig. 4. *Inro from the Florentine collection.*

A look at past exhibits, some of which became permanent, helps mark the interest of the museum for the Orient and in particular Japan.

We know that Japanese artifacts were exhibited in the previous location of the museum personally organized by Paolo Mantegazza in Capponi street before the collections were moved to Palazzo non finito in via del Proconsolo in the early 1920s (Rossi, 2014).

Even in the fascist/colonial period there were display cases with objects from the Orient (Leonore-Cecina, 1938) The Japanese objects, particularly from the collections of Branchi and Loria, were on the ground floor. Photographs document these exhibits with the note «China-Japan exhibits up until 4 November 1966, the day of the disastrous Florence flood». Indeed, these objects were recovered from the mud deposits and restored by the curators of that period. During archive research we found recently pictures with the date 26 November 1974 (Fig. 5).

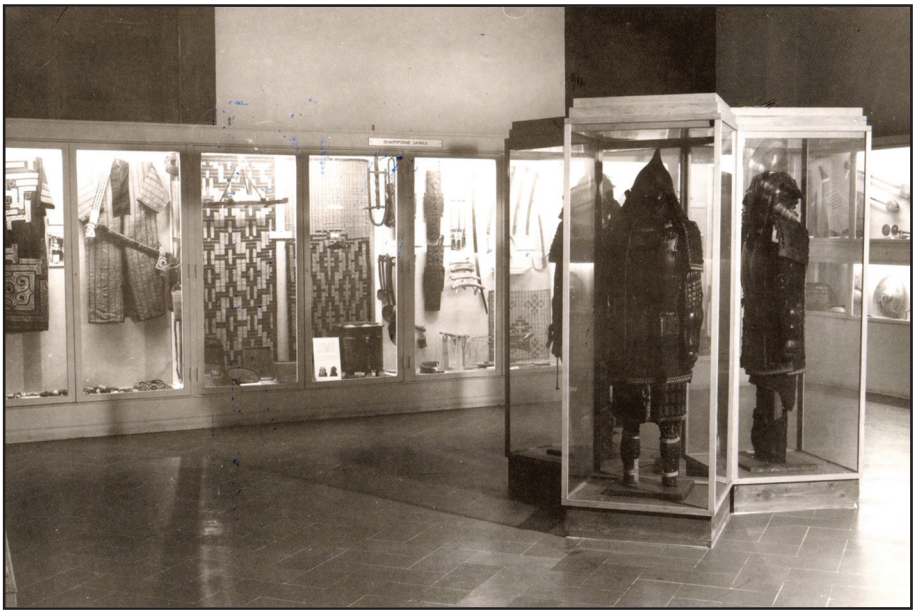

Fig. 5. J*apanese collections exhibit in 1974.*

The photographs also show that, after the disastrous flooding, rooms at the same ground floor were then restored and held the Japanese collections including exhibits of the Maraini collection, which was later moved to a dedicated room on the first floor (Fig. 6).

Fig. 6. *Ainu costumes on display in the room dedicated to Fosco Maraini's collection in present times.*

Conclusions

We hope to have provided with this brief report a glance at the history of the links between the museum and Japan. There exists also an historical connection between the museum of Giglioli's time with that of Maraini. The connection between these two scholars is evident in the recent publication of a selection of Giglioli's writings on Japan extracted from *Viaggio intorno al globo della regia pirocorvetta italiana Magenta negli anni 1865-66-67-68* (Voyage around the globe on the Italian corvet Magenta in the years 1865-1868). Indeed, Matteo Luteriani, founder of the editorial house Luni, dedicated this volume to Fosco Mariani who had encouraged him to publish the writings of Giglioli on Japan so that they would not be forgotten. Finally we want to underline how important is for us, curators of the Florentine museum, to dialog with our Japanese colleagues in regards to the museums collections. We sincerely want to acquire new information and elements to better understand the collection. Certainly we are extremely interested in learning about recent and ongoing attempts to valorize Ainu culture, both by collaboration with the Minpaku and with the new cultural center and museum dedicated to Ainu culture recently opened in Hakkaido.

Corresponding author: Francesca Bigoni: francesca.bigoni@unifi.it

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Of bears and boats: first digitalization of Ainu artifacts of the Anthropology and Ethnology Museum of Florence

SAVERIO BARTOLINI LUCENTI* ** GIULIA DIONISIO***
FRANCESCA BIGONI*** LORENZO ROOK*

Parole chiave: scansioni 3D, digitalizzazione museale, Giappone, etnografia.

Riassunto — Negli ultimi due decenni l'utilizzo di tecniche 3D *imaging* e tecnologie associate (stampa 3D, realtà virtuale o aumentata) ha guadagnato sempre più terreno in discipline legate al patrimonio culturale e a contesti museali. La raccolta di questo tipo di dati è diventato uno strumento valido in vari campi museologici: analisi e conservazione, fruizione del pubblico, condivisione con colleghi per studio e ricerca, restituzione virtuale per le comunità che hanno prodotto gli artefatti. In questo articolo descriviamo l'attività 3D scanning di due *iku-bashui* della collezione di artefatti Ainu del museo fiorentino. L'applicazione di questa tecnologia si è dimostrata particolarmente utile in questa tipologia di oggetti lignei, unici al mondo e caratterizzati da motivi decorativi intricati e vari, con alti significati simbolici.

Key words: 3D scan, museum digitalization, Japan, Ethnography.

Summary — Over the last two decades the use of 3D *imaging* techniques and their associated counterparts (3D printing, virtual or augmented reality environments) have gained ever more ground in cultural heritage disciplines and in museum contexts. The collection of 3D imaging data has become a valuable tool in various fields related to museology: analysis and conservation, public dissemination, opportunity of sharing such data with colleagues for study and research, virtual restitution for the peoples who produced the artifacts. In this article we report on the 3D scanning of two *iku-bashui* from the Ainu artifacts collection of the Florentine museum. The application of this technology is particularly useful for wooden objects of this type, characterized by intricate and diversified decorative motifs of high symbolic significance.

Introduction

The use of 3D imaging techniques (e.g., scanning, photogrammetry) and their associated counterparts (3D printing; virtual or augmented reality environments) has become more and more widespread in cultural heritage disciplines (*inter alios* Pavlidis *et al*., 2007; Bastir *et al*., 2019) and also in museal contexts (inter alios Wakowiak & Karas,

* Earth Science Department, Paleo[Fab]Lab, University of Florence, Italy.
** Natural History Museum, Geology and Paleontology section, University of Florence, Italy.
*** Natural History Museum, Anthropology and Ethnology section, University of Florence, Italy.

2009; Kęsik *et al.*, 2017; Pollalis *et al.*, 2018). Such an increasing interest from the numerous scientists and researchers in the field of cultural heritage was facilitated by the reduced cost of the instruments required to acquire digital data and by the ever more precise representation of the studied object that these scans are able to obtain. A number of works have investigated the suitability and even compared the accuracy of different scanning approaches on different objects (e.g. zoological specimens: Friedman *et al.*, 2015; sculptural archeological artifacts: Morena *et al.*, 2019; fossil bones: Kogan *et al.*, 2020). Of the different techniques, hand-held scanners are used in a wide variety of disciplines (in addition to those above: architecture: Maxwell, 2017; medicine: Adams *et al.*, 2015; Dessery & Pallari, 2018; forensic sciences: Larsson & Letalick, 2013; Wieczorek & Gorawska, 2017). One of the reasons for such diffusion is that hand-held scanners offer several advantages compared to stationary ones. For instance, hand-held scanners are highly flexible, work at a close range, are portable and efficient. Their shape and compact size allow them to enter and scan undercuts of large objects, but they are also suitable for imaging small objects, with complex geometries. Several works (Boehm, 2014; Kersten *et al.*, 2016; 2018) tested the accuracy of different of hand-held scanners, showing slight differences with the structure light projection systems. Nevertheless, recently hand-held scanners have become economically competitive with larger systems, which are indeed more precise but also more expensive.

Ethnological material heritage has unique characteristics. Artifacts are particular for the extreme variety of sizes, shapes, materials and the combinations of material. These objects often play a particularly sensitive role in museums. Many objects in their original contexts were conceived for everyday use, but also have ceremonial and ritual functions. They are often still intrinsically linked to very vital cultures, which claim their place in today's society. These cultures are increasingly sensitive to the appropriation of their traditions which often takes the form of material culture. In fact, many cultures use these objects to get back in touch with their tradition, which are heavily threatened by globalization. Ethical museums face a series of problems linked not only to conservation and use of collection, but also to the need of reestablishing links and collaborations with the communities from which the objects originally came from. The literature presents interesting examples of museums that have already experimented the use of these technologies for documentary and conservation purposes,

including collaborative anthropology (Trinchão Andrade *et al*., 2012; Hollinger *et al*., 2013; Enkhbat, 2015; Schmidt, 2016; Raimundo *et al*., 2018).

In 2018, two specimens of the Peruvian Pre-Columbian collection and of the Elio Modigliani's collection (respectively n. cat. 4107 and n. cat. 10767) were digitalized using Next Engine laser scan for teaching and explorative purpose. This tool, kindly lent by the Laboratorio di Geomatica per l'Ambiente e la Conservazione dei Beni Culturali (GECO) lead by Prof. Grazia Tucci (Dipartimento di Ingegneria Civile e Ambientale – UNIFI), is particularly suited for the digitalization of small- to medium-sized objects (Bigoni *et al*., 2019). Results were satisfactory yet such methodology does not allow you to register the texture of the object, which was added with subsequent photogrammetric methods. The present study represents the first application of digital imaging techniques using hand-held high-resolution scan technology with Artec Spider, that couples geometric and texture acquisition in a single pass, to a chosen ethnoanthropological sample of the Museum of Anthropology and Ethnology of Florence.

Iku-bashui

Iku-bashui are a particularly unique and interesting type of object. For details on *iku-bashui* in the collections of the Museum of Florence and in particular the Fosco Maraini collection, we refer here to the article in this volume by Bigoni and Roselli. Here we go into detail on the *iku-bashui* as an important artifact of the Ainu culture (Japan) to which Maraini dedicated great attention. In Maraini's collecting and research activities, he highlighted the complexity of these *iku-bashui* including their symbolism and meanings. Fosco Maraini is still considered one of the leading western expert on these artifacts. During his research in Japan, he published a detailed monograph (Maraini, 1942) which was reviewed a year later in *Monumenta Nipponica*, one of the oldest scientific journals published in Japan in English or other Western languages (Krakow, 1943).

Maraini's books on Japan are usually characterized by their narrative style and beautiful photographs. The photographs for the public were perhaps the focal point and Maraini was widely known for his activity as a photographer.

Instead, the monograph on *iku-bashui*, published in Italian, instead is strikingly different, systematic and scientific. With this work Maraini made available to Italian readers a quantity of information, at the time

was mostly available in widespread articles and books in Japanese. In this monograph Maraini underlined the uniqueness of the *bashui* in the panorama of human cultures: «It is interesting to note that over the world only the Ainu make use of these sticks» (note 1, p. 2). He defined the use of *bashui* in this way «we will immediately notice that to present the offering of wine to the gods the elders use a wooden stick, called *bashui*, which, held in the right hand, is titled with the tip in the cup and then moved in the air to disperse a few sprays of the drink here and there» (see Fig. 1).

Fig. 1. *Plate from Maraini (1942) showing the numerous different shapes and decoration of the iku-bashui. Note the number 24 correspond to one of the selected specimens for this study (n. cat. 32028).*

Maraini described two types of *bashui* (both of which are widely represented in his collection). «The *kike-ish-bashui*, for ceremonial use, usually made of sacred willow wood, manufactured expressly for the occasion in which it is needed and having on the upper face some shavings planed with a knife that remain attached to the base». And «the *Iku-bashui*, for common use, of any wood, without shavings, and mostly finely carved on the upper face».

Maraini combined different source of information: Western literature on the subject, limited only to short mentions (John Batchelor, Neil Gordon Munro, George Montandon), the much more informative literature by Japanese authors such as Hiromichi Kono, Kindaichi Kyosuke and Sugiyama Sueo, and the explanations obtained directly

from the Ainu, in particular Nitani Kunimatsu, and also many *ekashi* who had hosted him during his visits to the villages. Maraini recognized that the subject was very complicated and that above all the symbolic aspect, so richly expressed in the decorations of the sticks, still needed much study. He therefore set the research on a large amount of material and on a process of comparative analysis of the decorations. His study materials compared with his own already extensive collection, with other Japanese collections included the artifacts in the Museum of Sapporo.

Materials and methods

In this contribution we use digital scan technology to two *iku-bashui*, (n. cat. 32028 and n. cat. 32049). The scanned objects come from the Maraini Collection of the Anthropology and Ethnology Museum of the University of Florence. The first *iku-bashui* was chosen with a reference to the bear, a central theme of Ainu mythology and rituality. The second *iku-bashui* recalls, through the motif of the boat, the activity of fishing, essential for the livelihood of the Ainu villages on the coast. We have traced the descriptions of these two *iku-bashui* in the publication by Maraini (1942): n. cat. 32028 is described as a «*Bear head* (*Kamui Marapto Sapa*) in front of a cup, given to him as a present by Sueo Sugiyama» (Fig. 1), and n. cat. 32049 as «a representation of the boat» (in Maraini, 1942 a picture of the artifact in fig. 104 n. 11).

3D Scan and the protocol used

Tridimensional data were acquired with high-resolution blue light technology Artec Spider Scan held at the Earth Science Department of the University of Florence. The technology of this scanner is based on structured light (using a speckle pattern methodology) based on blue LED technology. Considering the relatively restrict linear field of view (between 18.0 x 14.0 cm and 9.0 x 7.0 cm) this scan is suitable for the detection of objects of small size. Indeed, its maximum resolution is 0.1 mm and the accuracy around 0.05 mm, with a maximum speed of acquisition of 8.0 fps. The texture of the scanned object is acquires using a 1.3 MP camera, which takes color pictures every chosen interval (default value 1 fps).

Models were generated using the native software (Artec Studio 14 Professional), following the standardized workflow process outlined in the user documentation (Artec Group, 2020). Specimens were placed

on a rotating plate with low reflectivity, for a better acquisition of the 3D surfaces (Fig. 2).

Fig. 2. *Scanning process of the two selected specimens. The objects were placed on a rotating plate covered with paper with low reflectivity, to facilitate the base removal procedure. Different passes in different orientation were made in order to get a full coverage of the object.*

In order to obtain the complete rendering of the external decoration of the *iku-bashui*, the selected specimens were scanned in different passes with significant overlap covered in the different scans. These methods also facilitate the automatic base removal procedure. The scans, once cleaned were aligned using a minimum of three homologue points, chosen by the user. Despite the flattened shape of *iku-bashui*, which sometimes affects the ability of the software correctly aligned margins, the alignment of 3D scans was successful. The aligned scans underwent three registration processes (rough serial, fine and global registration, all following default settings: geometry and texture; surfaces used as key frame: 0.3; distance between adjacent features: 5 mm) to merge different coordinate systems of points into a single, shared one. After cleaning the scans with the outlier removal algorithm, the polygonal 3D mesh was created with the Sharp Fusion tool (using resolution of triangulation grid: 0.2 mm). As the automatically generated resolution was satisfactory, e.g., correctly preserving all the decoration of the upper surface, no user manipulation was needed. Finally, texture was applied to the 3D mesh using the texture atlas method (4096x4096)

which generates a single texture file linked to the mesh. Once this protocol is over, the obtained 3D objects were saved as a geometry-only stereolithography file (STL) and a geometry-and-texture Polygon File Format with vertex color (PLY) directly from Artec Studio 14.

Discussion

The resulting reconstructions of the selected specimens are shown in Figs. 3-6. The used methodology acquires the texture and geometries of the objects with remarkable detail.

Figs. 4 and 6 show such particulars in both n. cat. 32028 and n. cat. 32049, from the nostrils and mouth of the bear (n. cat. 32028) to the net on the side of the boat (n. cat. 32049). Compared to the methodology employed by Bigoni *et al.* (2019), Artec Spider and the associated software allow a more rapid and defined acquisition of the specimens in fewer passes and procedures, compared to the previously used protocol. Thus, such a technique facilitates the effort of museum's collections digitalization. Undoubtedly, the powerful and effective tools, like these high-resolution scanners used here, grant numerous opportunities to expand the normal exhibition. For instance, three-dimensional objects can be used in virtual and remotely accessible online tours. General parameters of such files can be easily adjusted, just like for raw pictures, to highlight portions, details and features otherwise hidden or hardly visible. Note that several portion of the sculpturations of the scanned *iku-bashui* can be better identified in the geometry-only visualizations (Figs. 3-6). Relevant opportunities for museum exhibitions offered by modern technology is the implementation of 3D printing of the scanned specimens. For instance, the tiny objects could be made at larger scale to make features more visible. 3D printing grants also provide the opportunity to show an object too fragile or precious to be kept out of a showcase, thus allowing visitors of all ages to touch them or to insert them as part a museum itinerary for visually impaired people.

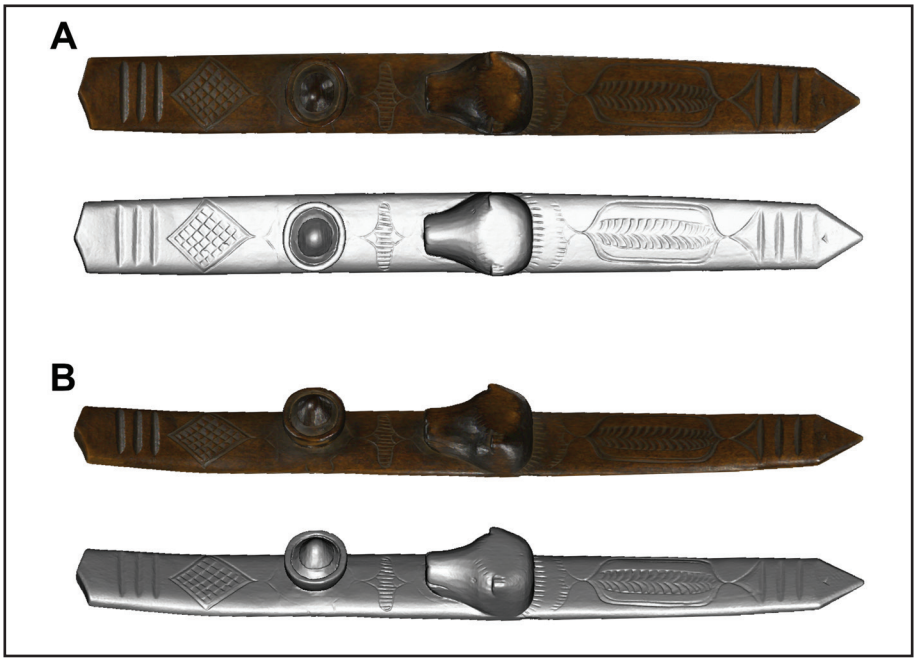

Fig. 3. *Different visualizations of the generated mesh after the scan of the bear iku-bashui (n. cat. 32028), in both texture rendering and simple mesh. A, in dorsal view; B in dorso-lateral view. Note the detailed pattern on the surface evident in both models.*

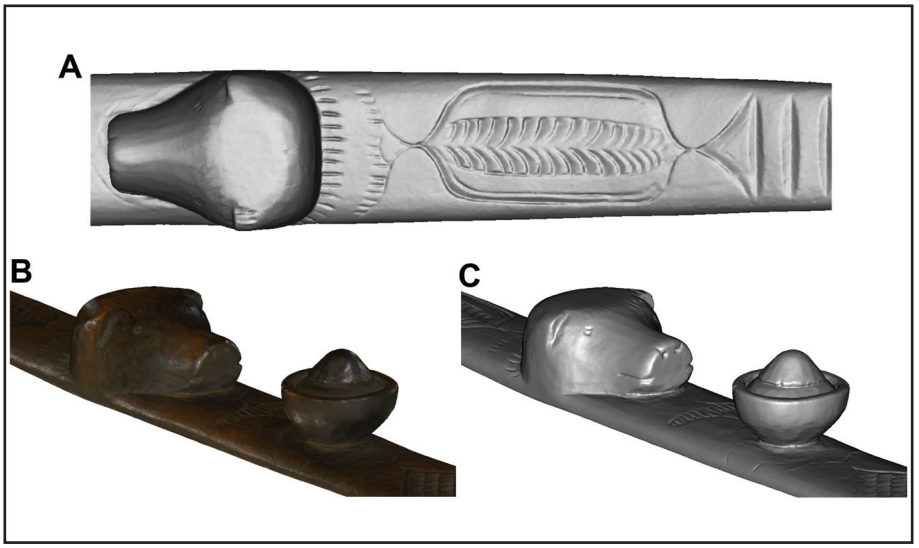

Fig. 4. *Detailed views of the pattern and sculpturing of n. cat. 32028 captured by the scans and reproduced on the mesh, both in texture rendering and simple mesh. A, in dorsal view; B-C comparison of the lateral view of the head of the bear and of the cup (probably the ritual one used during the Iyomante).*

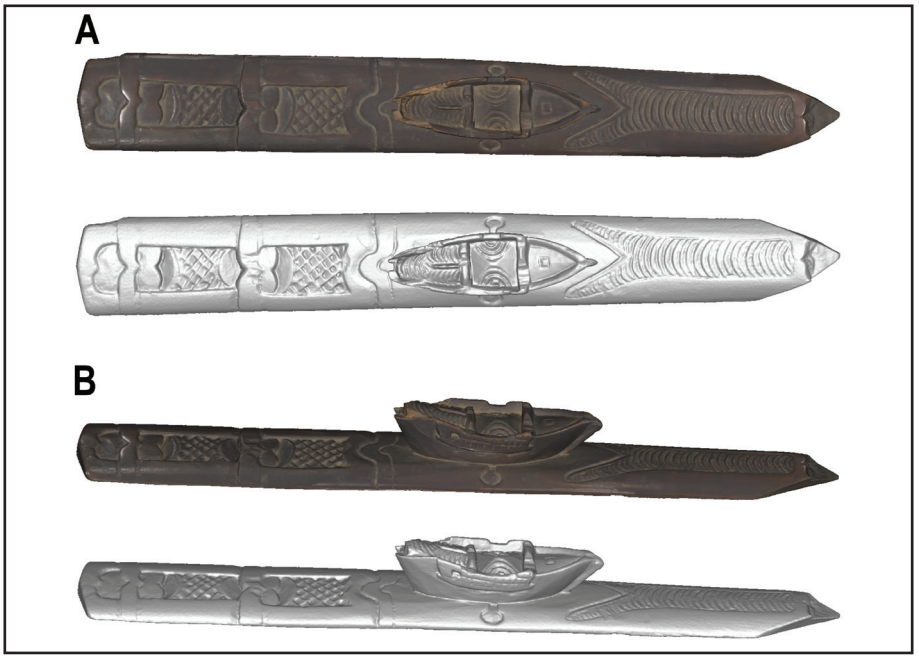

Fig. 5. *Different visualizations of the generated mesh after the scan of the boat iku-bashui (n. cat. 32049), in both texture rendering and simple mesh. A, in dorsal view; B in dorso-lateral view. Note the detailed pattern on the surface evident in both models.*

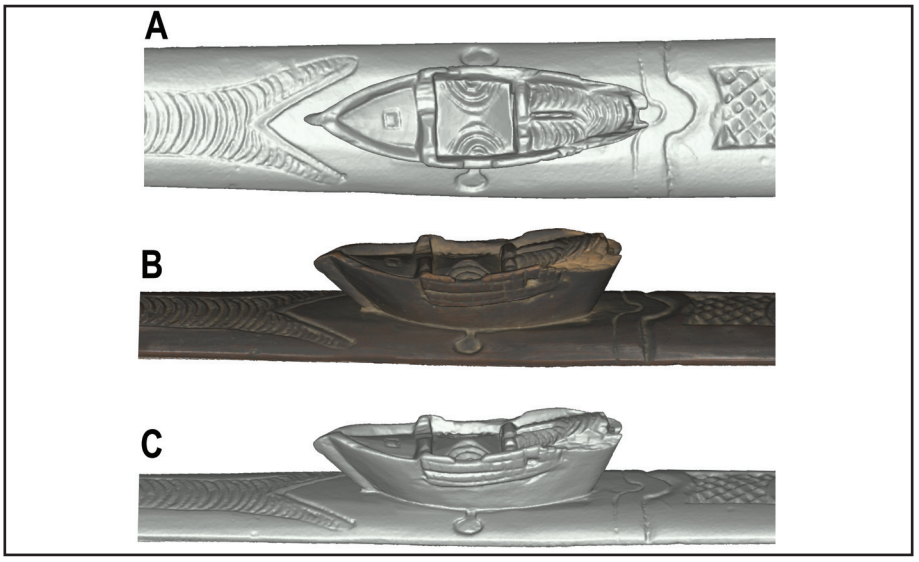

Fig. 6. *Detailed views of the pattern and sculpturing of n. cat. 32049 captured by the scans and reproduced on the mesh, both in texture rendering and simple mesh. A, in dorsal view; B-C comparison of the lateral view of the side of the fishermen's boat.*

Conclusions

Our use of high-resolution scanner Artec Spider has allowed us to achieve excellent results with a potential range of use of the information obtained that touches various aspects: analysis and conservation, public dissemination, sharing digital data with colleagues for study and research, virtual restitution for the peoples who produced the artifacts. The application of this technology has proved particularly useful for this type of wooden object, characterized by intricate and diversified decorative motifs of high symbolic significance.

«What do the objects and their information in the museum mean to us? […] People who are the original owners might use this information to create a new local material culture» (Nobayashi, 2016, 95). This is one of the crucial aspects of the museum's mission as a place for creating culture and build stronger communities. This function is fundamental for the communities close to the museum's headquarters, but also for those apparently distant to which the museum is indelibly linked by the objects it houses.

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Corresponding author: Saverio Bartolini Lucenti: saverio.bartolinilucenti@unifi.it

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History of human culture reflected in beads: the Bead Research Framework

KAZUNOBU IKEYA*

Parole chiave: network sociali, rivoluzione cognitiva, storia culturale.

Riassunto — Questo studio presenta un modello di ricerca finalizzato a comprendere la storia culturale delle relazioni tra gli esseri umani e i vaghi. L'autore ha raccolto informazioni su materiali utilizzati, metodi di produzione e ruoli sociali dei vaghi utilizzati nel mondo. Stabilire una narrazione storica attraverso questi artefatti può essere un approccio importante per ricostruire la storia di *Homo sapiens*. Più specificamente questo tipo di ricerca ha importante significato negli studi sulla rivoluzione cognitiva che è avvenuta nella storia umana ed è testimoniata dal commercio di perline prodotte da conchiglie, pietre, vetro e altri materiali, e, attraverso di esse, dalla formazione di networks sociali. I seguenti tre aspetti suggeriscono un modello di ricerca che può chiarire la storia e lo stato attuale del rapporto fra popolazioni e vaghi: materiali naturali e artificiali, tecniche di perforazione e di assemblaggio fra materiali diversi, e vari ruoli sociali. Nella storia della cultura dei vaghi, si può osservare la bellezza espressa in materiali e tecniche, e la presenza di networks sociali costruiti attraverso la condivisione del valore estetico. Questi network sono risultato di commerci e reti di distribuzione sviluppati in ragione della percezione comune che i vaghi sono oggetti belli che corrispondono a differenti parametri estetici nelle società del mondo.

Key words: social network, cognitive revolution, cultural history.

Summary — This paper aims to present a research framework for understanding the cultural history of relations between humans and beads. The author has collected information related to materials used, production methods, and social roles of beads used around the world. Establishing a history of beads can be one approach to developing the history of *Homo sapiens*. More specifically, research on beads is meaningful for investigating the cognitive revolution that has taken place in human history. Trade in beads made of shells, stones, glasses, and other materials, is informative about the formation and development of social networks through beads. The following aspects suggest research frameworks to elucidate the history and the current state of relations between people and beads: natural and artificial materials, techniques to make holes in bead materials, techniques to connect one material to another, and various social roles. In the history of bead culture, one can observe the beauty identified in materials and techniques, social networks built through aesthetic value as a result of trade and distribution developed because of the perception that beads are beautiful objects, and different aesthetic senses in societies around the world.

* National Museum of Ethnology, Osaka, Japan.

Introduction

Beads might be regarded as modern human beings' first works of art (Allen *et al*., 1998), created and spreading worldwide approximately 100,000-120,000 years ago (Dubin, 1987; Vanhaeren *et al*., 2006; Zilhao *et al*., 2010; Kadowaki, 2020). The following explanation first defines beads as materials into which a hole is made, which are, then mutually connected with a string (Ikeya, 2018b, 14). The materials are wide-ranging, including nuts, plant seeds, animal teeth and bones, seashells, ostrich eggshells, minerals such as stones, gold, and amber, glass, clay, paper and plastics. Bead crafts have used various shapes, from lines such as necklaces to surfaces such as bags. They are used not only as accessories for beautification. Beads are said to have played social roles as symbols of wealth, assertions of social dignity, signals of group identity, and as reflections of other status (Allen *et al*., 1998; McBrearty and Brooks, 2000; Stevenson and Stewart, 2000; Brumm *et al*., 2017; Ikeya, 2018b).

Among past studies of prehistoric humans, paintings such as the Lascaux cave paintings in France are well-known 20,000 years ago (National Museum of Nature and Science, 2016). Few studies of the past have been undertaken to develop a human history based on accessories and beads that are allegedly older than those paintings (Ikeya, 2020b). Past research on beads of the world, including *The worldwide History of Beads* (Dubin, 1987), has primarily investigated the history of glass beads. Those studies have specifically undertaken the description of individual cases of beads from geographical and historical perspectives, without noteworthy attempts to analyze such individual cases comprehensively.

The author has perceived as an issue the understanding of how beads seen in all times and places have been related to people from two perspectives: one is the history of approximately 120,000 years from the time when the creation of beads began, up to the present; the other is the geospatial space called the Earth, on which beads have been used. As the first step to this goal, this article was compiled to present a research framework for understanding the cultural history of relations between humans and beads. The author has collected information related to the materials and production methods used to make them and information elucidating the social roles of various beads that have been used around the world (Ikeya, 2018a; Ikeya, 2018b).

Human history and Beads

The approximately 300,000-year history of modern human beings can be categorized into three revolutions: cognitive, agricultural, and industrial (Harari, 2014). In the past, several studies have been done to assess the revolutions of farming and livestock breeding and the Industrial Revolution (Bellwood, 2004). People have come to produce food stably through farming and livestock breeding and have achieved mass production of food through industrialization, which has facilitated food supply activities for the entire human population, which has been growing rapidly. Few researchers, however, have directly argued for recognition of a cognitive revolution in the past (Harari, 2014). Cases marking a cognitive revolution include origin myths for the world and its peoples (Goto, 2017).

A cognitive revolution was achieved through changes in the brain of *Homo sapiens* during the Paleolithic era some 70,000 years ago. Changes helped humans not only memorize what was in front of them, but also imagine what was not in front of them by connecting information in the brain and creating stories such as myths. At the same time, they became able to describe to others what was not readily visible by sharing information using words (Harari, 2014). Such a cognitive revolution brought about various goods such as accessories, languages, dance, music using instruments, paintings, and more myths related to various topics. Among them, beads used as accessories have been excavated at archaeological sites, of which the ages have been identified. The beads which are considered as the oldest date back approximately 120,000 years. In other words, they do not correspond to the age of cognitive revolution argued by Harari, and were instead created in the eras pre-dating the paintings and other artifacts from approximately 30,000 years ago.

In conventional research, human behavior of the mid-Stone Age has been drawing attention to help clarify human history. Beads particularly were created approximately 120,000 years ago. They have been discovered worldwide. They are therefore regarded as marker materials for the beginning of the symbolic behavior of humans (McBrearty and Brooks, 2000). Information about beads is found in the permanent exhibitions at the Smithsonian National Museum of Natural History in the US, for instance. The origin of the beads has been revised to «from 75,000 to 100,000 years ago» (Fig. 1).

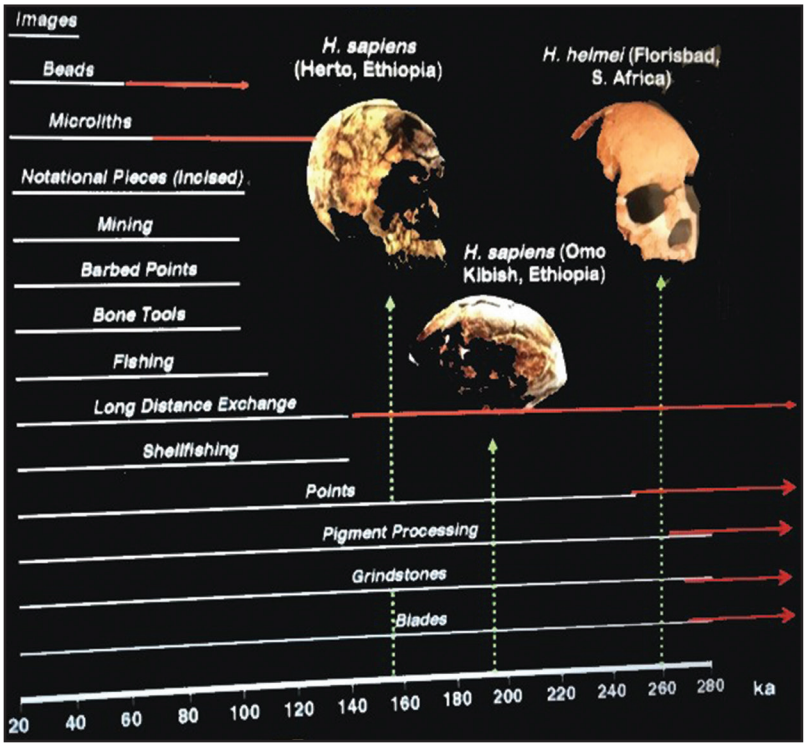

Fig. 1. *The revolution behaviors among African peoples in the middle stone ages (Smithsonian Museum of Natural History) (Photo: Ikeya).*

This figure presents also that the use of bone tools and fishery began in this period. Many questions, however, remain unanswered. They include the question of what factors caused the creation of beads and other crafts after 100,000 years from the birth of *Homo sapiens*. At the same time, beads have been discovered at a number of locations in Asia, to which humans spread after migrating from Africa. Whether these emerged independently or as part of cultural diffusion will be a subject of discussion.

Subsequently, paths such as «shell roads», «glass roads» and «stone roads» were created in the world as distribution channels of beads (Ikeya, 2018b). Along shell roads, cowries attract attention (Fig. 2). Through the African and Eurasian continents and Oceania, multiple shell roads existed for the transport of cowries. Demand for these shells was particularly high in Cameroon and Nigeria in West Africa and in the Congo and Ethiopia in Central Africa. Cowries were rare in the nearby Atlantic. Therefore, they were transported great distances from the Indian Ocean. Cowries have been used for various products such

as the chairs of the kings of the Bamileke in Cameroon, and masks and hats in the Kuba Kingdom in the Congo. In Ethiopia, these shells were incorporated into the life of various ethnic groups. Particularly, numerous cowries are stitched on children's backpacks. The Oromo people hang these bags on walls in their houses (Fig. 3). In New Guinea, dog whelk and other shells are used together with cowries to decorate statues of deities.

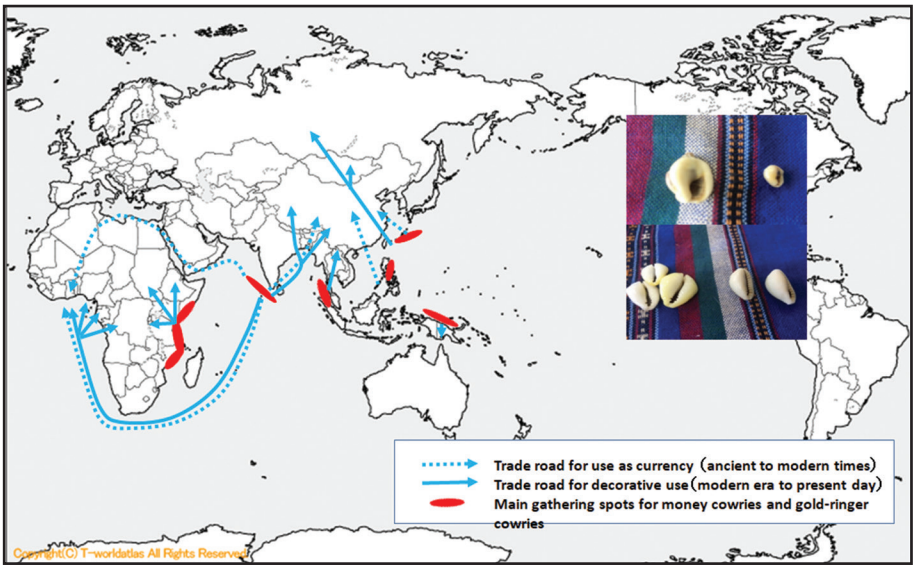

Fig. 2. *Cowries roads in Asia and Africa (source Ikeya, 2018a).*

Fig. 3. *A bead bag made in Japan.*

The bead roads include roads of stone, iron, glass, and other materials in addition to the shell bead road. Glass beads particularly were used as goods for exchange when contacting ethnic groups living in regions that were unknown to Europeans during the Age of Discovery(Coles and Budwig, 1997, 20). Glass beads helped connect people in remote regions in Africa, Asia, and South America to Europe, the center of civilization at the time(Dubin, 1987; Ikeya, 2018b). This represents the formation of the so-called world system, passed down to the global distribution of glass beads in modern society.

Three Frameworks to Understand Beads

This section presents research frameworks to help understand the history and present of relations between people and beads.

Materials

Several terms describe beads: glass beads, seed beads, and bead bags. Humans have used beads made of various materials. The total number has yet to be identified. They, however, are divisible between natural materials and artificial materials. Natural materials include plant seeds such as Job's tears and watermelon seeds, shells such as cowries and dog whelk, eggshells such as those of ostrich eggs, and the teeth of jaguars, dolphins, and monkeys (Ikeya, 2018b). Materials representing regional characteristics include hornet heads, arapaima scales, and snake bones, which are interesting. Stones such as carnelian and jade cannot be neglected despite the laborious work necessary for their collection and processing. Carnelian was used in Indus Valley civilizations (Endo, 2020). Jade was used in Japan during the Jomon period after long transportation from its production sites (Ikeya, 2018b). In addition, amber, coral, agate, lapis lazuli, and other materials have been used according to demand in different regions.

A major artificial material is glass. Glass production presumably originated in western Asia (Allen, 1998, 85). Today, the use of glass is spread to every corner of the world. In Venice, Italy, for instance, glass production techniques have been passed down only on the island of Murano. In Japan, too, the glass industry survives to this day (refer to Ikeya, 2020a). Another artificial material is iron. Iron beads are used in common by livestock farmers in the northern region of Kenya and the northern region of Namibia (Ikeya, 2018b).

Connecting beads requires strings and threads. For this purpose, plant fiber unquestionably comes in handy. This, however, is also

difficult to preserve as an archeological resource. In addition to plant fibers, tendons of large wild animals were taken out, connected like a string, and used for beads. The San people, who are hunter-gatherers in the Kalahari Desert, also use the tendons of gemsboks (large antelope-like animals) (Ikeya, 2018b).

As these examples indicate, widely various materials have been used depending on regional conditions. Not only were bead materials used also to connect beads; animal and plant materials that are difficult to preserve are also used. Bead researchers must devote attention also to their presence.

Technique

Bead techniques are divisible into techniques to make holes in beads and techniques to connect beads. Whereas information about puncture techniques is indispensable for production at production sites of materials such as stones and glass, information about connecting beads is more important at the stone and glass consumption sites. Bead craft techniques develop from lines to surfaces. In the history of beads in Japan, flat bead crafts such as those in ancient Egypt were developed into bead bags in the modern era (Fig. 4).

Fig. 4. *Leather bag covered cowries beads of the Oromo people in Ethiopia (Photo: Chikage Oba).*

Descriptions of puncture tools and materials, how to use them, and who uses them are fundamentally important for analyses. Bones of steenboks captured in traps are used for the ostrich eggshells used

by the hunter-gatherers of the Kalahari Desert(Ikeya, 1996; Hitchcock, 2012). Among the Yoruba people in Nigeria, bead craftspeople have ruled since the kingdom era. They currently use glass beads made in China, but purportedly produce hats, walking sticks, and other items covered with beads before delivering them to regional leaders (Ikeya, 2018b).

In technical, descriptions of traditional bead techniques in communities and understanding the formation and development of professional groups into which bead craftspeople would gather are some research themes. Detailed descriptions and comparisons of cases on the island of Murano in Venice (glass), India and Myanmar (stones and glass), ancient Japan (stones), and other places are required (Ikeya, 2018b).

Social connections: exchange and trade, color and shape selection criteria, and decoration methods

Distribution of beads helps determine research frameworks that vary depending on a spatial range of distribution such as local, regional, and global stages. Many plant and animal materials, for instance, are distributed at the local level. Among shells, some cowries (money cowry and gold-ring cowry) and amber produced in Europe are distributed regionally. Glass beads have been traded globally from ancient to modern and contemporary times (Ikeya, 2018b).

Beads connect a region to another through trade, which requires the determination of exchange rates and identification of traders for the study of the conditions of people's trade. In the literature from the 17th century, Dutch exchanged glass beads and sheep when they met the native Khoikhoi people in Cape Town (Fig. 5). The Ainu exchanged glass beads made in China and fur (Otsuka, 2020; Saito, 2020) (Fig. 6). In ancient Japan, stone that became bead material was produced in the Izumo region, after which it was transported to the Kinki region where beads were used and traded.

How beads are related to society varies depending on the condition of people's occupations. The following explains this point according to four categories: hunting and gathering, livestock and plant farming, kingdoms and emirates, and modern civilized society. First, hunter-gatherer societies viewed from a global perspective indicate that, in each society, glass beads are used in the first contact with outsiders (Ikeya, 2018b). In hunter-gatherer societies, people have received glass beads through first contact with outsiders. In the 7th to 18th centuries, not only hunter-gatherer societies, but livestock farming societies were

also not attracted to currencies. They had preferences in bead colors and shapes in each regional community. In the case of the San people in Africa, whereas the use of ostrich eggshells decreased as glass beads were brought in from the outside, the economic value of ostrich eggshell beads as folk crafts has been increasing in recent years (Ikeya, 1996; Hitchcock, 2012). In the case of Ainu, beads made of natural materials might have decreased in the past as a result of the introduction of large glass beads among the people (Otsuka, 2000).

Fig. 5. *A copperplate print representing the way glass beads owned by Dutch people were being traded with ivory and sheep owned by Khoikhoi herders in Cape Town (Ikeya, 2018a, 47).*

Fig. 6. *Glass beads of an Ainu woman.*

For livestock and plant farmers, the use of beads has played the role of signifying the identity of groups within an ethnic group or in distinguishing ethnic groups in some communities. In the Zulu society in South Africa, for instance, people allegedly visualized differences among regional groups based on differences in the color arrangements of glass beads (Ikeya, 2018). In the Dinka society in South Sudan, men wear a corset made of glass beads. The colors of a Dinka man's corset indicated the age group to which he belonged (Beckwith and Fisher, 2008, 26).

In kingdoms and emirates, beads are used to assert a social hierarchy, from a king to a commoner. The Yoruba society in Nigeria had traditional chiefs whose hats, walking sticks, and other belongings were produced by local bead craftspeople (Fig. 7) (Drewal and Mason, 1997; Ikeya, 2018b). In the kingdoms of Cameroon, cowries obtained in long-distance trade were used to decorate the king's chairs. The beads used for all such chairs differed from the beads used by commoners. Finally, in modern civilized society, beads are used particularly in various rituals. Examples in the contemporary Japan include prayer beads used in funerals and pearl necklaces worn by brides in weddings (Ikeya, 2020a). In recent years, bracelets worn casually by men and women's silk necklaces are also commonly observed in contemporary Japan.

Fig. 7. *The bead craft produced by Yoruba people in Nigeria (Photo: Kazunobu Ikeya).*

Summary: Humans' «Cognitive Revolution» and Pursuit of Beauty

This article aimed to present a research framework for understanding the cultural history of relations between humans and beads. The author collected information related to individual materials used, production methods, and social roles of beads used around the world. The results clarify the following two findings.

1) Restoring a history through beads can be one approach to developing the history of *Homo sapiens*. More specifically, research of beads is meaningful for investigating the cognitive revolution that has taken place in human history, the trade of beads made of shells, stones, glasses, and other materials, and the formation and development of social networks through beads. For example, shells transported from the coasts of the Mediterranean and the Atlantic have been discovered in ruins of sapient humans that date back 120,000 years. These are thought to have been introduced through long-distance trade among different groups. Moreover, the aesthetic appeal and scarcity of beads underlying such trade likely promoted the formation of social networks. In other words, they do not correspond to the age of cognitive revolution argued by Harari.

2) The following aspects suggest research frameworks to elucidate the history and the current state of relations between people and beads: natural and artificial materials, techniques to make holes in bead materials and techniques to connect one material to another, and various social roles. Particularly, relations between beads and societies vary depending on the conditions of people's occupations and societies. These are categorized into four: hunting and gathering, livestock and plant farming, kingdoms and emirates, and modern civilized society.

Based on the points raised above, in the history of bead culture, one can observe the beauty identified in materials and techniques, social networks built through aesthetic value as a result of trade and distribution developed because of the perception that beads are beautiful objects, and different aesthetic senses in societies around the world. Through these, a hypothesis used to explain the origin and development processes of human beauty will be presented in future research.

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Corresponding author: ikeya@minpaku.ac.jp

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RENDICONTI DELLA SOCIETÀ ITALIANA DI ANTROPOLOGIA E ETNOLOGIA

615ª Adunanza del 21 febbraio 2020
Assemblea Generale Amministrativa

La Riunione ha inizio alle ore 11,55 in seconda convocazione nella sede sociale di Palazzo Nonfinito. Presiede il Presidente P. Mannucci che dà la parola al Consigliere Amministratore M.G. Roselli. Vengono presentati alla ratifica dell'Assemblea il Bilancio consuntivo 2019 e il Bilancio preventivo 2020, accompagnati dalla relazione scritta dei Sindaci Revisori sullo Stato Patrimoniale della Società e sul Bilancio consuntivo 2019. L'Assemblea, dopo averne preso visione, approva all'unanimità il Bilancio. Si procede alla nomina dei Sindaci Revisori per l'anno 2020. Vengono riconfermati i Soci E.C. Lombardi, R. Tempestini e V. Serino.
Il Presidente ricorda all'Assemblea che il Consiglio Direttivo della Società quest'anno è in scadenza e che si deve procedere al suo rinnovo per il quinquennio 2020-2024, quindi, invita i presenti, a norma dell'articolo 8 dello Statuto vigente, a proporre una lista nella quale sia specificato il nome di due candidati alla carica di Presidente e 15 candidati come Consiglieri. Su proposta del Presidente viene posta in votazione una modifica dello Statuto consistente nell'abolizione della distinzione tra soci residenti e non residenti in occasione delle votazioni per il rinnovo del Consiglio. L'Assemblea approva all'unanimità.
La votazione dell'Assemblea, per scrutinio segreto, dà i seguenti risultati:
Votanti 15, per la Presidenza ricevono voti L. Sineo (12), C. Scarsini (10), G. Chelazzi (3), F. Bigoni (1). Per i Consiglieri: G. Stefania (14), P. Mannucci, M.G. Roselli, C. Scarsini (11), R. Biagi, F. Bigoni, M. Fabiano, R. Stanyon (10), L. Sineo (9), G. Dionisio, E.C. Lombardi (7), M.E. Frati, R. Tempestini (6), R. Paloscia, L. Pollarolo (5), L. Bachechi, F. Barbagli, M. Lari (4). Non vengono riportati i nomi dei soci che hanno ricevuto meno di 4 voti.
La lista proposta dall'Assemblea per il rinnovo del Consiglio Direttivo quinquennio 2020-2024 risulta così composta: per la Presidenza C. Scarsini, L. Sineo. Per i Consiglieri R. Biagi, F. Bigoni, G. Dionisio, M. Fabiano, M.E. Frati, E.C. Lombardi, P. Mannucci,

M.G. Roselli, R. Paloscia, L. Pollarolo, C. Scarsini, L. Sineo, R. Stanyon, G. Stefania, R. Tempestini.
Non essendoci altro argomento all'o.d.g. la riunione si chiude alle ore 13,00.

Dopo l'Assemblea del 21 febbraio 2020, a causa della pandemia virale che ha colpito la popolazione italiana e dei conseguenti provvedimenti governativi, non è stato possibile convocare le altre Adunanze in presenza della Società, con la cadenza prevista in Statuto. In una Riunione del Consiglio del 28.12.2020, svoltasi in streaming, si è deciso di dare seguito alle votazioni del nuovo Consiglio, da espletare nel febbraio del 2021, con modalità adeguate alla situazione di pandemia non ancora conclusa.

NORME DI STAMPA

1) La Rivista, organo ufficiale della Società Italiana di Antropologia e Etnologia, pubblica annualmente articoli inediti, notizie, necrologi, recensioni e i Rendiconti della Società.

2) Spetta al Direttore scientifico della Rivista, coadiuvato dal Comitato dei Revisori, formulare il giudizio sulla validità scientifica degli articoli e decidere sulla loro pubblicazione.

3) Il termine per la consegna degli articoli è fissato entro e non oltre la fine di giugno, quello per la consegna di testi relativi a notizie, recensioni e necrologi entro e non oltre il 30 settembre alla «Redazione della Rivista», e-mail: roscoe.stanyon@unifi.it; giulia.dionisio@unifi.it.

4) Gli Autori devono seguire le seguenti disposizioni: i testi dovranno essere inviati come allegati di posta elettronica, salvati in formato Word e dovranno rientrare nel limite massimo di 6000 parole, incluse le figure ed i riferimenti bibligrafici, e non essere superiori ad una lunghezza massima di 10 pagine. Normalmente si esegue una sola bozza di stampa che dovrà essere rinviata alla Redazione entro e non oltre 10 giorni dalla ricezione, pena la non stampa del lavoro.

5) Si accettano articoli sia in lingua italiana che in lingua inglese, purchè corredati, salvo note brevissime, da almeno due riassunti (non più di 300 parole), dei quali uno in lingua italiana. I riassunti dovranno essere preceduti dalle rispettive «parole chiave» (in un numero da 3 a 5).

6) Ogni autore deve indicare la propria affiliazione in forma generica (se prevista) e il proprio indirizzo di posta elettronica.

7) L'intestazione del lavoro nella prima pagina deve seguire questa forma: prima il titolo del lavoro in grassetto seguito da nome e cognome del o degli Autori scritti per esteso e in carattere maiuscolo, poi i riassunti in italiano e inglese preceduti dalle rispettive «parole chiave» ambedue in maiuscoletto; a piè di pagine verranno indicati gli Enti nei quali l'Autore o gli Autori lavorano o fanno parte.

8) Usare Times New Roman come font comune e solo una dimensione di carattere (12 pt) per l'intero contributo (incluso il titolo principale, le intestazioni, i sotto-titoli ed i riferimenti bibliografici). Utilizzare il corsivo per le parole in lingua diversa dall'italiano.
9) I simboli delle unità di misura vanno scritti senza punto finale.

10) Per quanto riguarda le citazioni di frasi di altri autori all'interno del testo utilizzare i seguenti simboli grafici « ».

11) Non includere tabelle o illustrazioni nel testo corrente (NB: i grafici sono illustrazioni). Salvare le illustrazioni individualmente e separatamente dal testo in un file a parte contrassegnando ciascuna con il proprio nome: es. McCarthy-Figura 1).

12) Se si utilizzano illustrazioni provenienti da altre fonti e non di propria produzione includere il possessore del copyright al termine della didascalia. Inviare le illustrazioni solo in formato elettronico, in alta risoluzione (minimo 300 dpi per le immagini senza testo, 1200 dpi per le immagini o tabelle contenenti testo). Il formato scelto per le illustrazioni è TIFF (non inviare le illustrazioni in formato JPG o GIF).

13) Le immagini a colori saranno stampate in bianco e nero nella copia cartacea del volume ma rimarranno a colori nel pdf che verrà inviato a ciascun autore ed eventualmente in open access per il download.

14) Assicurarsi di citare le didascalie nel testo corrente secondo le indicazioni riportate in parentesi (es. Fig. 1, Tab. 2, etc.) che saranno usate come riferimento per posizionare le immagini o le tabelle nel corso dell'impaginazione tipografica. La lista delle didascalie inerenti alle figure deve essere allegata alla fine del contributo.

15) Le didascalie devono essere redatte secondo i seguenti esempi: Tab. 1. *Classificazione del colore delle iridi;* Fig. 3. *Piroga monoxila.*

16) Le citazioni bibliografiche nel testo debbono essere in tondo e racchiuse entro parentesi tonda, devono comprendere il solo cognome dell'autore scritto per intero, seguito da virgola, l'anno di pubblicazione del lavoro, la pagina o le pagine (in tal caso unite da trattino), la tabella, la figura a cui si fa riferimento. Es. (Parenti, 1960, 130-143), (Mallegni, 1983, Tav. 7), (Corrain, 1987, 145 e Fig. 4).

17) Nel caso si citino all'interno del testo opere di più Autori, ci si limiterà a citare il cognome del primo Autore facendolo seguire dalla indicazione *et al*. in corsivo (Pardini *et al*., 1983, 5), ma nell'elenco dei Riferimenti Bibliografici si indicheranno i nominativi di tutti gli Autori.

18) Non si ammettono di regola note a piè di pagina.

19) Le opere citate o comunque inerenti al lavoro sono raccolte alla fine del testo sotto la dicitura Riferimenti Bibliografici, non vengono numerate e sono diposte in ordine alfabetico d'Autore, e a parità d'Autore in ordine cronologico. Solo i riferimenti bibliografici citati nel testo possono comparire nella lista bibliografica. Si prega di non usare alcun tipo di abbreviazione (riviste, collane etc.).E' possibile inserire le pubblicazioni in corso di stampa ma è necessario riportarne la data. L'intera voce

bibliografica della quale riportiamo alcuni esempi dovrà essere in tondo e in numeri arabi.

Riferimenti bibliografici (esempi):

Monografie (riportare tutti i titoli in corsivo):
- Cremaschi, M. 2000. *Manuale di Geoarcheologia*. Roma: Laterza.
- Conrad, G.W., Demarest, A.A. 1984. *Religion and Empire: The Dynamics of Aztec and Inca Expansionism*. Cambridge: Cambridge University.
- Braidwood, R.J., Braidwood, L.S. 1960. *Excavations in the Plain of Antioch I. The Earlier Assemblages, Phases A-J*. Oriental Institute Publications 61. Chicago: University of Chicago.
- Edwards, I.E.S., Gadd, C.J., Hammond, N.G.L. (a cura di). 1970. *The Cambridge Ancient History I.1. Prolegomena and Prehistory*. Cambridge: Cambridge University.

Articoli in riviste (non riportare i titoli dei contributi in corsivo):
- Cherry, J.F. 1981. Pattern and process in the earliest colonisation of the Mediterranean islands, *Proceedings of the Prehistoric Society*, 47: 41-68.

Capitoli di monografie:
- Beckman, G. 1988. Herding and herdsmen in Hittite culture. In: E. Neu, C. Ruster (a cura di), *Documentum Asiae Minoris Antiquae: Festschrift für Heinrich Otten zum*, 75. Wiesbaden: Harrossowitz: 33-44.

Pubblicazioni on-line:
- Knapp, A.B., Kassianidou, V., Donnelly, M. 1999. *Politiko Phorades: Excavations of a Bronze Age smelting site in Cyprus*. Internet Edition: http://www.scsp.arts.gla.ac.uk/Phorades/index.htm.

20) Tutti i contributi verranno sottoposti alla revisione da parte di almeno due referees.

21) Non sono previste spese di pubblicazione. Tutti i costi saranno coperti dalla Società Italiana di Antropologia e Etnologia.

INSTRUCTIONS TO AUTHORS

1) The Journal, official publication of the Italian Society of Anthropology and Ethnology, annually publishes original articles, news, obituaries, reviews and reports.

2) The scientific Director of the Journal with the cooperation of the Editorial Board will evaluate the scientific validity of the article and decide on its publication.

3) The deadline for the submission of the articles is fixed at no later than the end of June, while the deadline for the delivery of news, reviews and obituaries at no later than September 30 to «Editorial Staff of the Journal» at e-mail: roscoe.stanyon@unifi.it; giulia.dionisio@unifi.it.

4) The authors must comply with the following provisions: the texts should be as e-mail attachments and saved in Word. Papers, including figures, tables and references should not exceed 10 printed pages or about 6000 words. Normally only one gallery proof is sent to the authors which must be returned to the Editorial Office no later than 10 days after receipt, otherwise the article will be published as is.

5) The title page should include in the following order: Title in Bold font, name and family name of the authors in capital letters, Keywords and Summary, and subtitles should all be in small capitals.

6) Institutional affiliation and email should be supplied for each author.

7) The abstract or summary should be no more than 300 words. An italian summary will be supplied by the Editorial staff. From 3 to 5 keywords should listed before the abstract.

8) Please use Times New Roman as the font, 12 pt, throughout including titles and subtitles.

9) The symbols for measurement units must be written without a final period.

10) Quotations in the text should be set between the following symbols: « ».

11) Figures and tables should be sent separately from the text with a file name of the first author and figure number, for example: McCarthy-Figure 1.

12) If illustrations are not originals they must be accompanied by copyright permission. Illustrations should be in TIFF and have a minimum resolution of 300 dpi. Images with text should have a resolution o 1200 dpi.

13) Color illustrations will be printed in black and white in the hard copy of the journal, but will be in color in the pdf which will be supplied to each author and eventually on open access for downloading.

14) Figures and tables must be cited in the text within parenthesis, for example (Fig. 1, Tab. 2) etc. and will be used to place figures in the eventual publication. Legends to tables and figures should be in italics and collected at the end of the text and should be as follows: Tab. 1. *Classificazione del colore delle iridi;* Fig. 3. *Piroga monoxila.*

15) Any bibliographical references in the text must enclosed in parenthesis. They must include only the full surname of the author, followed by a comma, and the date of publication of the work, the page or pages, the table or figure to which one is referring. Here are some examples: (Parenti, 1960, 130-143), (Mallegni, 1983, Tav. 7), (Corrain, 1987, 145 e Fig. 4).

16) If the works of several authors are cited, indicate the name of the first author followed by the words «*et al.*» in italics (Pardini *et al.*, 1983, 5), but the list of references will indicate the names of all authors.

17) No footnotes are allowed.

18) The works cited or otherwise relating to the text are collected at the end of the text under the heading Bibliographical References, are not numbered and are arranged in alphabetical order of the authors, and by the same authors in chronological order. Use the full name of the journal, not an abbreviation. All references must be cited in the text.

Examples:

Books (titles in italics):
- Cremaschi, M. 2000. *Manuale di Geoarcheologi*a. Roma: Laterza.
- Conrad, G.W., Demarest, A.A. 1984. *Religion and Empire: The Dynamics of Aztec and Inca Expansionism*. Cambridge: Cambridge University.
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19) All submission will be reviewed by at least two referees.

20) There are no fees for publication. All costs will be covered by the Italian Society of Anthropoogy and Ethnology.

INDICE DEGLI AUTORI

INDICE DEL VOLUME CL — 2020

RENDICONTI

INDICI

Direttore responsabile: Dott. Piero Mannucci
Registrato presso il Tribunale di Firenze con Decreto n. 1045 del 27 ottobre 1955
Settembre 2020 Petruzzi Srl - Città di Castello

SOMMARIO

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