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Holistic Anthropological Research of Hvar Islanders, Croatia – From Parish Registries to DNA Studies in 33 Years

Pavao Rudan[1], Branka Janićijević[1], Veljko Jovanović[1], Jasna Miličić[1], Nina Smolej Narančić[1], Anita Sujoldžić[1], Lajos Szirovicza[1], Tatjana Škarić-Jurić[1], Lovorka Barać Lauc[1], Tomislav Lauc[1], Irena Martinović Klarić[1], Marijana Peričić[1], Diana Rudan[2] and Igor Rudan[3,4]

[1] Institute for Anthropological Research, Zagreb, Croatia
[2] General Hospital »Sveti Duh«, Zagreb, Croatia
[3] Department of Medical Statistics, Epidemiology and Medical Informatics, School of Public Health »Andrija Štampar«, Faculty of Medicine, University of Zagreb, Zagreb, Croatia
[4] Department of Public Health Sciences, Medical School, University of Edinburgh, Scotland, UK

ABSTRACT

The complexity of interactions between hereditary, environmental and cultural factors in determining human phenotypes is often underestimated in biomedical research. In this paper, we present 33 years of holistic anthropological research that was being conducted since 1971 in the island of Hvar, Croatia. During this period, detailed characterization of migrations, demography, isonymy, linguistic differences, anthropometric traits (head and body dimensions), physiological (cardio-respiratory) properties, quantitative and qualitative dermatoglyphic traits, radiogrammetric metacarpal bone dimensions and genetic traits (classical antigens, HLA diversity, DNA short tandem repeat -STR, mitochondrial DNA and Y-chromosome polymorphisms) was performed. The analysis of this large collection of data using both model-bound and model-free approaches showed that the complexity underlying human biological traits may be considerably greater than generally assumed, which has important implications for design of future studies into genetic determinants of complex traits.

Key words: *phenotypes, genetics, environment, culture, population structure, holistic approach, modelling, anthropology, island of Hvar, Croatia*

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Introduction

The complexity of interactions between hereditary, environmental and cultural factors in determining human phenotypes is often underestimated in biomedical research[1–4]. It can be most clearly demonstrated when a well-defined human isolate population is considered and multiple measurements of phenotypes, genetic characteristics and studies of environmental and cultural determinants are performed and analyzed[5–7]. Even in such small communities, where decreased variability in all those factors is presumed, it is often apparent how difficult it is to disentangle the effects of separate interacting factors and explain most of the variance in phenotypes of interest. The application of holistic analytic approach in anthropological research, as »model-bound« or »model-free« approach, can be very helpful in providing some important information about the continuity of interaction between population genetic characteristics (its gene pool) and a wide spectrum of environmental selective impacts[5–9]. Major advantages of holistic approach are the possibility to assess the main determinants of within- and between-population similarity or variability, and to estimate the dependence of population structuring processes upon the historical processes that favored or restricted gene flow. Some of the extrinsic influences that have the greatest importance in such interaction are cultural, economical, medical, political, religious and social.

Historically, the leading directions in research of biological diversity of human populations included ecological approach[10], population genetic approach[11,12], analyses of population structure[13] and studies of human population structuring through history[14–16]. However, the experience that we gained through a number of such studies made us aware of major differences between results of the assessment of population structure when using different types of traits[9,17–21]. Generally, some traits appear to be more resistant to the environmental effects, and therefore they may be considered current traces of ancient founding population characteristics. Conversely, other traits reveal a much greater rate of change under environmental, ecological and cultural impacts (e.g. selection, growth, migration and mobility, etc.), which all adds to the complexity and needs to be taken into consideration in interpreting the results. Sokal[22], for example, suggested with respect to genetic structuring of populations that the »*...differentiation is due not only to the geographically limited mobility of populations (isolation by distance) and the possible effects of selection but also to presumed long-distance movements of various populations in the past*«. Due to a variety of aforementioned reasons, it is logical that, in order to obtain the best possible understanding of the determinants and characteristics of population's structuring process and its standing genetic and phenotypic variation, a holistic approach to the analysis is essential. This approach takes most account of the fact that historical processes are laboratories in which human populations are created.

In this study, for purpose of obtaining holistic insight into determinants of genetic and phenotypic variation of the Hvar island's population, various measures of genetic distances, biological distances, bio-cultural distances and socio-cultural distances were calculated and interpreted. *Genetic distances* were estimated separately from 9 STR loci, 5 HLA class II polymorphisms, 10 classical antigens, mtDNA and Y chromosome polymorphisms and isonymy. *Biological distances* were analyzed based on biometrical traits such as body and head anthropometry, physiological traits, dermatoglyphic traits and radiogrammetric measurements on metacarpal bone dimen-

sions. *Bio-cultural* distances were estimated from data on migrational kinship during four periods in recent history. The kinship distance matrix was also developed based on isonymy. *Socio-cultural distances* included linguistic distances measured separately from basic and cultural vocabulary.

Materials and Methods

Choice of investigated population

Island isolates are among the most suitable populations for theoretical analyses of human population differentiation and structuring[23]. As some of the few persisting isolates among contemporary European human groups, rural populations of the islands of the eastern Adriatic in Croatia reveal some of the peculiarities that make them very suitable for such analyses[1,24]. Some of those characteristics include reconstructable population history, known migrations that have occurred during a very long time period, their continuing reproductive isolation and well-documented effects of various extrinsic events that, through generations, directly influenced their biological formation[1,25].

Figures 1 and 2 schematically present what is presently known about long-term and more recent population history of the islands in the Eastern Adriatic, Middle Dalmatia, Croatia, respectively. The village populations of those islands represent a well-characterized genetic isolates. (Over 100 publications describe the population history, migration patterns, genealogical reconstruction, characterize biometrical traits, disease prevalences and environmental and socio-cultural characteristics of these populations[19,25] and www.inantro.hr). The population substratum was being formed until 800 AD by admixture of proto-Illyrians, Illyrians, Greeks and succeeding Romans, and by Croatian (Slavic) immigrants from the 5th century AD who spoke *čakavian* dialect which became dominant, leading to »Croatization« of the island from the 9th century. Population superstratum was formed during 16–18th century AD. It was formed by the Croatian population from the mainland who fled the Balkans peninsula in the wake of the Ottoman expansion. Those immigrants spoke *štokavian* dialect of Croatian language. The subsequent tendency towards inbreeding in each village has been influenced by geographic isolation, political (*»Paštrović«)* privileges given to residents of certain communities and socio-cultural reasons. All three factors added to population sub-structuring as they prevented gene flow both within and between the villages. Finally, the population *adstratum*

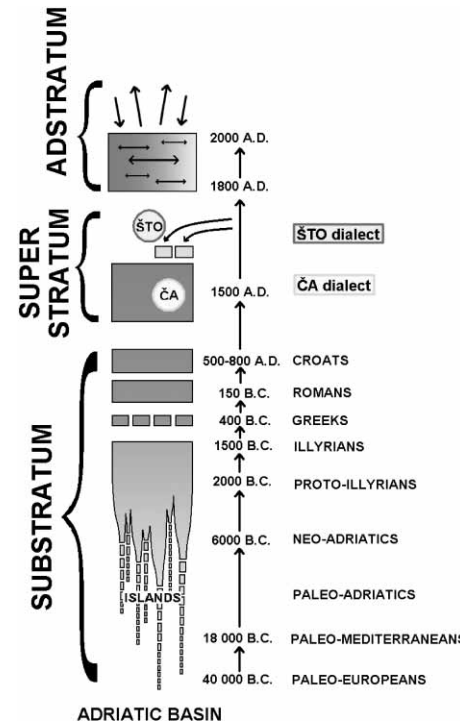

Fig. 1. Brief schematic presentation of long-term population history of island populations in middle Dalmatia, Croatia.

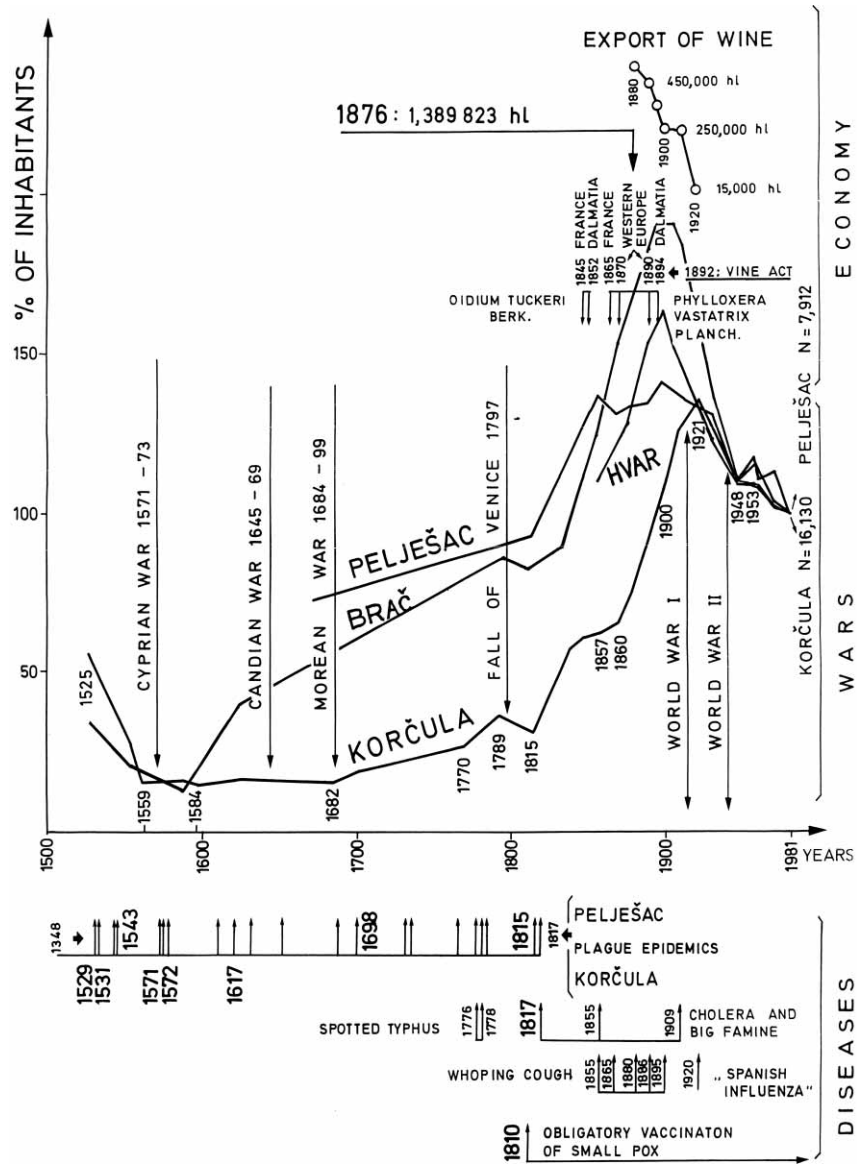

Fig. 2. Number of inhabitants (% of the number of inhabitants in 1981) on the islands of Korčula, Brač, Hvar and the peninsula of Pelješac) from 1525. There are two bottleneck effects – first one after plague epidemics in 14th century and the second at the beginning of the 20th century primarily caused by non-random emigration due to the economic crisis caused by the »wine act» in 1892 and vineyard devastation (wine export during 5 decades decreased by almost 100%). During Cyprian, Candian and Morean wars the inhabitants from the coastal areas immigrated extensively. The increase in the number of inhabitants from the beginning of the 19th century is among others a consequence of obligatory vaccination against smallpox, cultivation of potato as a new crop and increased wine production and export for almost 1.5 millions of hectoliters per year.

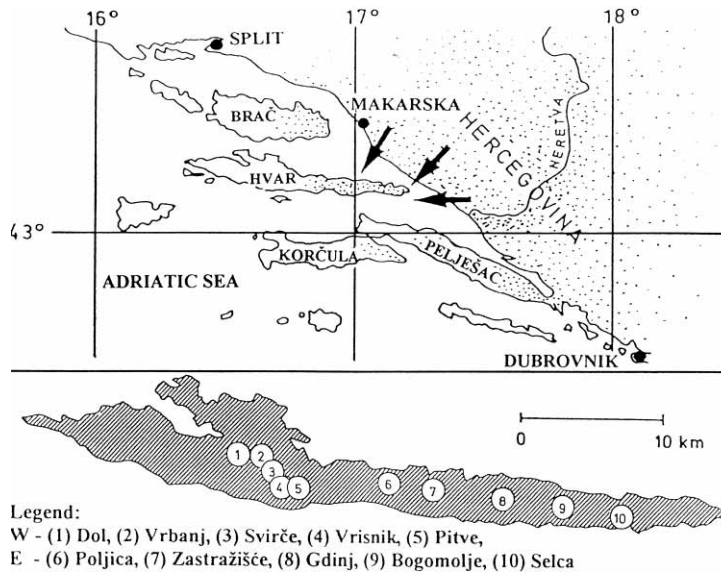

Fig. 3. Middle Dalmatian area (islands of Brač, Hvar and Korčula and Pelješac peninsula) and geographic location of 10 settlements on the island of Hvar, Croatia. Arrows present the paths of population immigration during the 16th and 17th century.

was formed within the past two centuries (Figure 2) by infrequent immigration from and non-random emigration to the mainland[25–27].

Figure 3 shows the location of 10 studied isolate settlements of the island of Hvar, Croatia.

Figure 4 shows demographic history of the isolate populations of the island of Hvar studied in this paper, presented as the number of inhabitants during the period from 1857 up to 1981 (as the majority of field research was performed between 1978–1979). It is common to nearly all villages that an increase in population size was observed until the year 1900, after which there has been a gradual decline in population size.

Sources, nature and collection of the phenotypic and personal history data

The first anthropological field investigation of the island of Hvar was carried out in 1971 by Rudan[24,28–30]. In 1978 and 1979, holistic anthropological investigations have been continued by the staff of the Department of Anthropology of the Institute for Medical Research and Occupational Health of the University of Za-

TABLE 1
DISTRIBUTION OF THE EXAMINEES BY 9 VILLAGES OF THE ISLAND OF HVAR

Village	Examinees	% of total
1. Dol	97	21.8
2. Vrbanj	120	17.8
3. Svirče	149	26.5
4. Vrisnik	98	38.0
5. Pitve	58	23.3
6. Poljica	40	29.6
7. Zastražišće	130	37.7
8. Gdinj	130	42.2
9. Bogomolje (+10. Selca)	112	44.6
Total	934	28.9

greb[31–33], which from 1992 became the Institute for Anthropological Research. The sample consisted of 934 examinees of both sexes, aged 18–65, who were chosen at random from a population of 10 villages and covered 28.9% of their population. The distribution of the examinees by villages and share in a total village population is presented in Table 1. Throughout the years, additional data were collected from above sample (such as blood sampling for the DNA analysis during 1990's, described later in the text).

The data collected for each examinee in each village included the place of birth of the examinees and their 2–3 generation pedigree (this was used for computation of migrational kinship for four important periods in recent history: 1857–1891; 1892–1913; 1914–1940; 1941–1981). Surnames of the examinees and their ancestors were obtained in all cases (which was used for isonymy kinship estimation). Basic and cultural vocabulary (for the estimation of linguistic distances), anthropometric head and body dimensions, physiological (cardio-respiratory) properties, quantitative and qualitative dermatoglyphic traits (digitopalmar complex) and metacarpal bone radiographs were all used to compute distance matrices between villages. Information on population census between 1857 and 1981 was obtained (this was used for estimation of demographic distance, based on dissimilarities in percentage of population increase/decrease from the initial values for the period between 1857 and 1981).

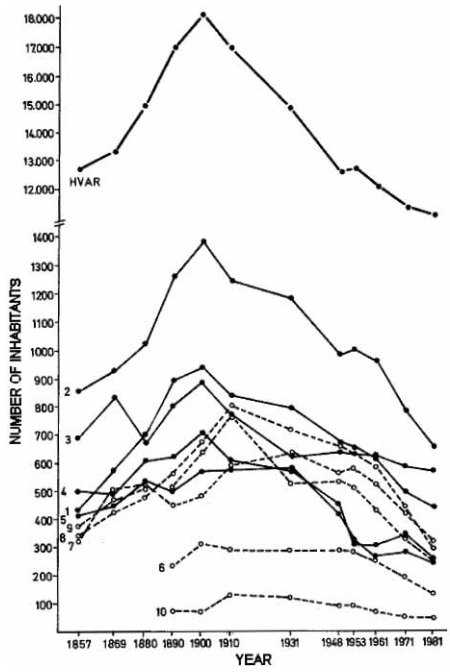

Fig. 4. Demographic history of the studied isolate populations of the island of Hvar, Croatia presented as the number of inhabitants during the period 1857–1981 (Note: for village codes see Figure 3).

All the measurements were made according to the IBP recommendations[34], »Textbook of Applied Biological Anthropology – Physiological Methods I«[35], and »Genetic methods I and II«[36,37]. The methods of data collection and measurements of the island's population are presented in detail in the papers of Sujoldžić et al.[33,38], Rudan[39,40] and Smolej Narančić et al.[41,42]. Table 2 reviews the analyzed traits, with a short description of methods of computation of inter-population distances from each trait and the reference to the description of applied methodology.

Body dimensions (BODYD-M, BODYD-F) included the measurements of a total of 24 traits: height, sitting height, leg length, upper leg length, lower leg length, total arm length, upper arm length, forearm length, biacromial diameter, transverse chest, antero-posterior chest, biiliocristal diameter, bicondylar humerus, bicondylar femur, chest circumference, abdomen circumference, upper arm circumference, forearm circumference, upper leg circumference, lower leg circum-

TABLE 2
LIST OF ANALYZED TRAITS: CODE FOR EACH TRAIT, SHORT DESCRIPTION OF THE TRAIT AND THE WAY THE INTER-POPULATION DISTANCES WERE CALCULATED, THE SOURCE OF THE METHODOLOGY

Trait code	Short description	Source of methodology (ref.)
KM	Shortest likely road distance between villages of the same island – geographic distance between analyzed populations.	___
KINP 1–4	Migrational kinship estimated from parent-offspring birthplaces separately for male (M) and female (F) examinees – calculated for the four different periods: 1892–1913; 1914–1940; 1941–1960; and 1961–1980.	43, 44
KINP-ISON	Kinship among villages estimated from the distribution of surnames as a qualitative data	45
DEMD	Demographic distance estimated from the dissimilarities in percentage of population increase/decrease from the initial values, for the period between 1857 and 1991	46
LINGD-BAS LINGD-CUL	Linguistic distances estimated by Hamming's similarity measures for 106 words of basic (B) and cultural (C) vocabulary.	47
BODYD (M/F)	Distances in body dimensions based on 24 measured traits* and calculated separately for male (M) and female (F) examinees according to Mahalanobis' D^2.	17, 48
HEADD (M,F)	Distances in head dimensions based on 14 measured traits* and calculated separately for male (M) and female (F) examinees according to Mahalanobis' D^2.	17, 48
PHYSD (M,F)	Distances in physiological (cardio-respiratory) properties based on 8 measured traits* and calculated separately for male (M) and female (F) examinees according to Mahalanobis' D^2.	17, 48
DERMD-QN (M,F)	Distances in quantitative dermatoglyphic properties based on 3 measured traits* and calculated separately for male (M) and female (F) examinees according to Mahalanobis' D^2.	17, 48
DERMD-QL (M,F)	Distances in qualitative dermatoglyphic properties based on 3 measured traits* and calculated separately for male (M) and female (F) examinees according to Hiernaux Δg.	17, 49
BONED (M,F)	Distances in radiogrammetric metacarpal bone properties based on 3 measured traits* and calculated separately for male (M) and female (F) examinees according to Mahalanobis' D^2.	48–51
GEND-STR	Genetic distances between villages determined from frequencies of 9 STR DNA loci*#	52–55
GEND-HLA	Genetic distances between villages determined from frequencies of 5 HLA class II polymorphisms*#	56–58
GEND-ISO	Genetic distances between villages estimated from the distribution of surnames as a qualitative data	59
GEND-CLASS	Genetic distances between villages determined from frequencies of 10 classical antigen systems*, 3 serum proteins* and 10 erythrocyte enzyme systems*, calculated as standard E^2	60, 61

* – specific sub-traits forming the entire »trait variable« are listed in further text;
# – calculated in a reduced sample for a total of six villages

ference, triceps skinfold, subscapular skinfold, abdomen skinfold and body mass. *Head dimensions* (HEADD-M, HEADD-F) included the measurement of 14 traits: head length, head breadth, minimal frontal width, bizygomatic diameter, bigonial diameter, morphological face height, nose height, nose breadth, mouth width, lip thickness, ear length, ear breadth, inter-orbital width and head circumference[32,62]. *Physiological properties* in males (PHYSD-M) and females (PHYSD-F) included the measurement of 6 traits of lung volumes and flow rates (FVC, FEV_1, PEF, $MEF_{25\%}$, $MEF_{50\%}$, $MEF_{75\%}$) and arterial blood pressure (systolic and diastolic)[31,41].

The analysis of *dermatoglyphic traits* included both quantitative and qualitative ones. The five *quantitative traits* were total papillary ridge counts on fingers (TRC), ridge counts between the digital triradii on palms (rc a-b, rc b-c and rc c-d), and size of the »atd angle« measured on both hands in males (DERMD-QNM) and females (DERMD-QNF). The 7 *qualitative traits* included the type of fingertip patterns (arch, ulnar loop, radial loop or whorl), the presence of patterns and triradii in the palmar areas (thenar/I, II, III and IV interdigital, and hypothenar), and pattern intensity index (PII) in males (DERMD-QLM) and females (DERMD-QLF)[30]. The measurements of *metacarpal bone dimensions* were performed on postero-anterior right and left hand's radiographs according to the Barnett and Nordin[50]. They included the measurements of three traits, i.e. total bone length, diaphysis width and medullar channel width of the left second metacarpal bone in males (BONED-M) and females (BONED-F).

Analyses of genetic distances based on HLA, classical antigen polymorphisms and isonymy

From blood specimens obtained from the examinees, genetic distances were es-

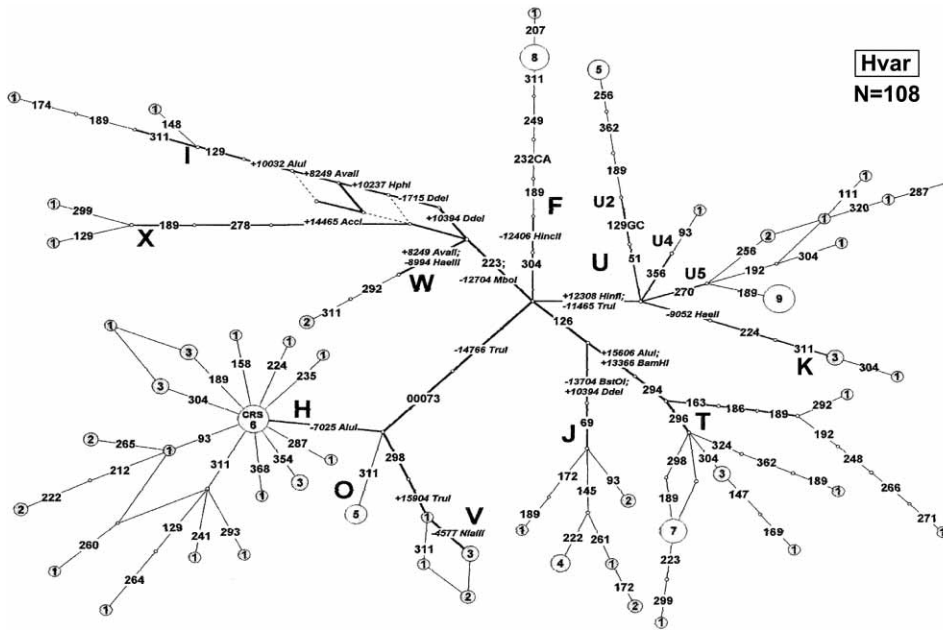

Fig. 5. Distribution of mtDNA haplogroups in 108 examinees from Hvar island.

timated separately from HLA class II polymorphisms, classical antigens and isonymy (see Table 2). The methods of data collection, handling and analysis for HLA were described by Grubić et al.[58], for a classic set of antigens by Borot et al.[61], and for isonymy by Relethford[59]. Genetic distances computation between the isolate populations was based on: differences in DRB1, DRB3, DRB5, DQA1 and DQB1 HLA class II polymorphisms (GEND-HLA); differences in the frequencies of 21 polymorphic system: classical antigens (including 10 genetic systems: ABO, Rh, MN, Ss, Duffy, Kidd, P, Kell, Colton and Lutheran), 3 serum proteins (haptoglobin, third component of complement, properdin factor B) and 10 erythrocyte enzyme systems (adenosine deaminase, esterase D, acid phosphatase 1, adenylate kinase 1, 6-phosphogluconate dehydrogenase, glucose 6-phosphate dehydrogenase, malate dehydrogenase, lactate dehydrogenase A and B, and phosphoglucomutase 1) (GEND-CLASS); and isonymy, as described by Roguljić et al.[62] (GEND-ISO).

Recent analyses of genomic information (STR, mtDNA and Y chromosome polymorphisms)

*Genetic distance*s between the isolate populations were also determined based on more recent genomic analyses, in two ways. Matrices of genetic distances were computed according to Schriver et al.[52], based on differences in D3S1358, wWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820 STR loci (GEND-STR).

Additional analyses have also been performed using genomic information obtained from the analyses of mitochondrial DNA (mtDNA) and Y chromosome markers in a sample of 108 and 92 examinees recruited during the 1990's, respectively. The methodology of these analyses have been excessively described for mtDNA in

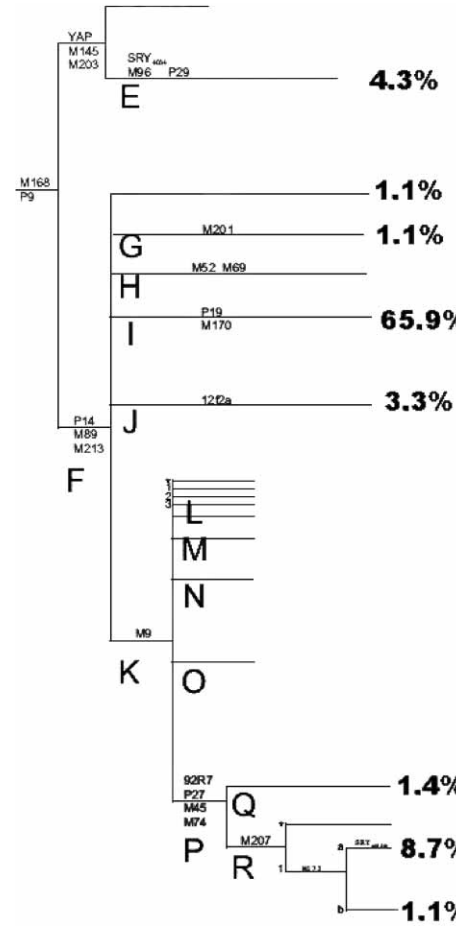

Fig. 6. Distribution of Y-chromosome haplogroups in 92 examinees from Hvar island.

papers by Tolk et al.[63,64] and for Y chromosome by Barać et al.[65]. The interpretation of the obtained distribution of haplogroups in the population (see Figures 5 and 6) in terms of population evolution, structuring and long-term and recent migrations has been offered in other publications[63–65]. For the purpose of this study, however, measures of distances and kinship have been computed based on mtDNA and Y-chromosome information. These four matrices only included the 6 villages in which the analyses have been

performed, and it should be noted that the estimates were based on a largely reduced sample (N of approximately 100) in comparison to distance and similarity matrices based on other traits[63–65]. Therefore, the obtained matrices were used to assess the applicability of isolation by distance model (independently of other traits) in a »model-bound« analysis, but were excluded from the »model-free« analysis due to incompatibility with other data. It should also be noted that the predictive statistical power of the isolation by distance model, when applied to the matrices based on DNA analyses obtained from a limited sample and in a limited number of villages, is much lower than for the other traits. Therefore, it is possible that the proportion of variance explained by the model could be substantial for some of these traits, but it still fails to reach statistical significance, as was the case with genetic distances computed based on Y-chromosome polymorphisms (see Table 3).

Concomitant research on inbreeding effects with biomedical implications

As an addition to studies of population biological variation, described up to this point, the concomitant research was being carried out into inbreeding effects on a number of biomedically relevant quantitative and qualitative traits. Such studies can provide useful information on the underlying genetic architecture of those traits, e.g. are they likely to be oligogenic or highly polygenic, is the variation maintained mainly by common or rare genetic variants, and are those mainly recessive or dominant. Those results could also be supportive of the analyses of biological information, and this is why we will present very brief results of that research with relevance to this paper.

Individual inbreeding coefficients were computed for each study participant on the islands of Hvar, Brač and Korčula. This was based on pedigree information on 4 ancestral generations (five generations where these occurred over a similar timeframe), recorded during the initial field work and supplemented by a study of parish registries stored in local churches. The individual inbreeding coefficients (*F*) were then computed according to Wright's path method[66]:

$$F = \sum_{1 \to c} (1/2)^{(n_i + m_i + 1)}$$

where m and n refer to the number of paths from a common ancestor, and c refers to the number of common ancestors. Then, the genealogical inbreeding coefficient for each village was computed as the average of all individual F values. To further support these estimates, F was calculated from isonymy as suggested by Relethford[59], and mean values were derived for each village on several studied islands. Estimates based on isonymy are generally thought to be positively biased, and so to provide an upper bound for F. The estimates were also supported by analyses of inbreeding at the village level from serogenetic polymorphisms[67].

Unlike the retrieved biological information, effects of inbreeding were estimated for prevalence of 10 most common late-onset complex diseases, blood pressure, cog- nitive dysfunction, nephrolithiasis and oro-dental health status, all of which have been obtained by separate field research in 1999 and 2000. Inspection of local medical records revealed that the 10 most commonly reported medical conditions with adult onset were coronary heart disease, stroke, cancer, schizophrenia, epilepsy, uni/bipolar depression, asthma, adult-type diabetes, gout and peptic ulcer. Specific diagnostic criteria were established for each of these 10 conditions following those presented in the 16th edition of Merck's Manual (for details see[68]). Two medical doctors, who were unaware of the inbreeding status of each in-

dividual, inspected the medical records and recorded whenever appropriate diagnostic criteria were met. The doctors visited each village on Hvar island between March and October 2000 and reviewed medical records of all inhabitants in collaboration with local general practitioners, who typically had lived in community for a number of years and were familiar with each patient's history. Diagnoses were supported wherever possible by medical records from consultant specialists at the University Hospital in Split. Disease prevalence was first investigated by comparing the prevalence of disease between villages grouped by the level of inbreeding: high, moderate or low. Disease prevalence rates were standardized by sex and age to the total population of 4 villages included in the study, using 10-year age intervals and direct standardization.

All the data are available upon request at the Institute for Anthropological Research in Zagreb, Croatia.

Model-bound and model-free approach to analysis of the data

There are two main approaches to analysis of population structure using biological trait measurements. Relethford and Lees[69] stated that the first one, »the model-bound approach«, implies direct application of various models of population structure to the observed variation within the population. The second one, »the model-free approach«, is used to analyze the structure and intensity of interpopulation variability without assuming any model. The former approach represents an explicit, and the latter an implicit use of genetic models. The results of both of these approaches were already presented and/or compared in analyses of the population structure of Eastern Adriatic isolates, e.g. on the islands of Hvar[9,20] and Korčula[17–19], Pelješac peninsula[5,17,19], Brač[6,21,70] and Pag islands[7] as well as Selška Valley in Slovenia[71].

For a total of ten villages listed in Table 1 (with exception of DNA-based studies, performed in examinees from 6 villages), we computed 36 pairwise estimates of migrational kinship, linguistic, biometrical, genetic as well as geographic distances. In the *model-bound approach* to population structure analysis, the relationship between kinship coefficients estimated from migrational data and geographic distances was assessed according to Malecot's isolation by distance model (i.e., regression analysis)[43,72]. The model states that the coefficient of kinship (relative or conditional) varies with geographic distance according to the expression

$$r = (1–L)ae^{–bd} + L \qquad (1)$$

where a is the expected within population kinship, b is the rate of decrease, d is the shortest likely road distance between villages (km), and L is the lower limit for conditional kinship in the examined region. L value is mainly used as a balancing term with little direct biological interpretation, although Morton[44] believed that it should represent a measure of drift from founder to contemporary populations. Biological, linguistic and genetic distances (D^2) increase with geographic distance according to the formula

$$D^2 = a\,(1–e^{–bd}) \qquad (2)$$

where a represents the value to which the square of distances asymptotically approaches when geographic distance increases; b is the rate of increase in biological distance, and d is shortest likely road distance between villages[73]. We estimated a and b in Eq. (1) and in Eq. (2) by nonlinear regression and tested the fit of the model by analysis of variance of residuals around the regression with a significance criterion of $p<0.05$[74]. The authors are aware that, due to the difficulties in determination of significance criteria when working with matrices, somewhat stricter p-value might have been used. However, our matrices are based on total

numbers of examinees that we consider sufficient for considering this p-value significant (Table 3).

In the *model-free approach* to population structure analysis, the correlation matrix of distance matrices was computed separately for males and females, taking into consideration the differences in migrational patterns by gender[23].

Correlation coefficients between distance matrices were calculated according to the test of matrix correspondence that was initially developed by Mantel[75], with the use of correlation extensions of that method as suggested by Smouse et al.[76]. The pattern of correlation between measures of migrational kinship, linguistic, biological, and genetic distances, as well as geographic distances among villages, was assessed by principal component analysis (PCA), which yielded the eigenvalues and percent of total variance explained by each factor. In order to improve interpretability[77], the extracted raw principal components were submitted to direct oblimin rotation with $\delta = -1$, and the results of rotated factor analysis were presented separately in males and females, along with the eigenvalues and percents of explained variance obtained through PCA.

Results and Discussion

The aim of this study is to reveal the underlying complexity of variation in human phenotypes, genotypes and bio-cultural traits in an isolate island population through holistic analysis using model-bound and model-free approach. Those measurable population characteristics have been shaped through the continuous interactive processes resulting from the constant interaction of population genetic characteristics and a wide spectrum of environmental influences through history. It will be shown that different traits responded in quite different ways to a totality of those impacts, presumably due to relatively large differences in genetic architecture of those traits. Therefore, we hypothesize that a holistic approach to analysis of multiple traits in a well-defined isolate population with similar environmental impacts could demonstrate remarkably different responses of specific traits to the totality of impacts that cause population structuring. Such analysis could point to a highly polygenic architecture of some traits and more oligogenic/monogenic of the others, as well as to the differences in interaction with environmental factors. The differences of genetic architecture of studied traits should be revealed as a considerably different response to similar environmental impacts through history[26].

Model-bound approach (Malecot's isolation by distance)

The results obtained through the application of model-bound approach, in this case Malecot's isolation by distance model, are presented in Table 3. From a total of 29 matrices regressed to geographic distance measured in kilometers, for 2 of them the regression was highly significant (BODYM and KINP 2, $p<0.001$). For 15 more traits the regression was significant at the level of $p<0.05$, while for 12 other traits no significant regression to the model could be demonstrated. Such finding implies that Malecot's model is generally very descriptive for the Hvar population, and that formation of population genetic structure was quite well predicted by the model from the majority of traits, but not from all of them. One of the factors that could strongly contribute to such positive result is certainly the geographic shape of the island and location of the villages: they are located approximately on the straight line, and long-range migrations were less likely than the short-range ones. This made island of Hvar an ideal population in which to test

TABLE 3
PARAMETERS OF MALECOT'S ISOLATION BY DISTANCE MODEL. TRAITS ARE LISTED IN DESCENDING ORDER, ACCORDING TO PERCENTAGE OF TRAIT VARIANCE EXPLAINED BY THE MODEL (R^2). (NOTE* THAT FOR MATRICES DERIVED FROM GENOMIC ANALYSES, THE SAMPLE WAS LIMITED AND THE POWER TO DETECT STATISTICAL SIGNIFICANCE LARGELY REDUCED)

Traits	a	b	R^2	p
BODYD (M)	0.0951	0.0928	0.6350	<0.001
KINP 2	0.0234	0.0473	0.5630	<0.001
HEADD (F)	0.0764	0.1068	0.5086	<0.01
BODYD (F)	0.1036	0.1111	0.4887	<0.01
HEADD (M)	0.0825	0.1106	0.4861	<0.01
KINP 4	0.0101	0.1069	0.4485	<0.01
GEND-HLA*	0.0544	0.1889	0.4447	<0.01
DERMD-QN (M)	0.2569	0.0958	0.4172	<0.01
GEND-Y*	0.0142	0.0620	0.3470	ns
KINP-HLA*	0.0075	0.0238	0.3180	<0.05
BONED (F)*	0.6526	0.0769	0.2489	<0.05
GEND-STR	0.2185	0.3564	0.2432	<0.05
KINP 3	0.0137	0.0278	0.2358	<0.05
DERMD-QN (F)	0.2240	0.3072	0.2218	<0.05
LINGD-CUL	0.5196	0.2156	0.2028	<0.05
KINP 1	0.2564	0.0416	0.1862	<0.05
DEMD	0.2355	0.2467	0.1857	<0.05
LINGD-BAS	0.3280	0.2990	0.1573	<0.05
BONED (M)	0.4023	0.2952	0.0838	ns
KINP-DNA	0.0958	0.0007	0.0710	ns
KINP-Y*	0.0840	0.0111	0.0650	ns
GEND-CLASS	12.1480	53.6773	0.0311	ns
KINP-ISON	0.1158	0.0199	0.0233	ns
PHYSD (M)	0.2004	61.9501	0.0031	ns
KINP-CLASS	0.0883	0.0074	0.0010	ns
GEND-MT*	0.1686	0.0008	0.0010	ns
KINP-MT*	0.0778	0.0002	0.0000	ns
GEND-ISO	0.6444	0.5719	0.0000	ns
PHYSD (F)	0.4991	314.4334	0.0000	ns

* Based on more recent, considerably smaller sample, described in detail in references 52–58

this model, which was considerably less predictive in, e.g., Brač and Pag islands[6,7].

In addition, Figure 7 shows the graphical illustration of the non-linear regression for a measure of similarity and a measure of distance with the highest regression scores. The measure of similarity with the highest regression score (shown by the upper graph) was the migrational kinship calculated for the pe-

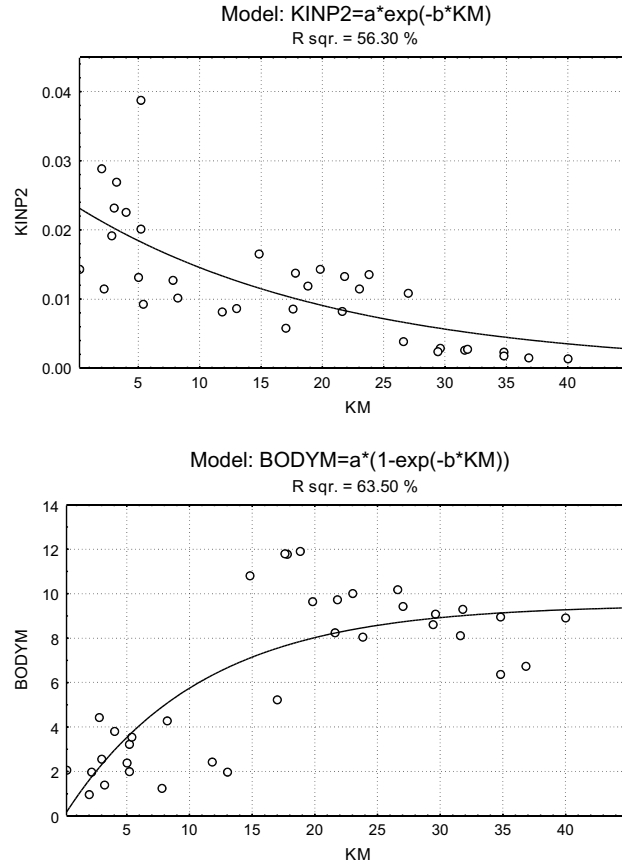

Fig. 7. Measures of similarity and distance with the greatest portion of variance explained by isolation by distance model – examples of high predictive power of the model for migrational kinship and body measurements (individual dots represent village pairs).

riod 1892–1913 (KINP-2), and 56.3% of its variance was explained by this model. The lower graph shows the regression of anthropometrical body dimension distances (BODYM) to the geographic distance, with 63.5% of the variance explained. Although this trait appears to show a good »fit« to the model, one might note the potential existence of so-called »shift effect« at the distances greater than 15 kilometers[78]. It indicates that the processes of biological and so-called biocultural or cultural homogenization were not always happening in the same direction.

When discussing these results, one should stress that the most commonly used measure of variation among human groups is Wright's F_{ST}[79], which is, in Relethford's[59] interpretation, simply the measure of the average squared distance of populations from a central point. However, although the measures such as F_{ST} provide information about the degree of variation among populations, they say nothing about the pattern of such variation[59]. Malecot's isolation by distance model[43,72] enables a theoretical prediction of the relationship between genetic kinship

and geographic distance among observed populations. In our study, kinship was estimated from migrational data from 1892, and the whole computation process was based on the theoretical assumption that systematic pressure originates from the same gene pool, i.e. that immigration to the island came from a single population[9]. Bearing in mind the limitations to this assumption (see ethnohistory paragraph!), the obtained results for kinship in males and females need to be taken with a degree of caution.

Another fact which cannot be neglected is that the obtained results indicate that the isolation by distance model is adequate for the evaluation of (micro)evolutionary processes based on certain traits, but not on others, which was one of the key theses stated in the opening paragraph of this study. Significance of regression ranges from very high (for anthropometrical body and head dimensions and migrational kinship) to very low (matrices based on monogenic traits, genomic haplotypes and physiological properties).

Such finding is in line with the hypothesis that the main determinant of the applicability of the isolation by distance model in Hvar island's population is variable-specific, rather than gender-related. That is contrary to the results of some previous studies of eastern Adriatic islands, e.g. of Korčula[17–19], Brač[18,70] and Pag[7]. In all of those studies, as a rule, biological distances and migrational kinship in females revealed better »fit« to the isolation by distance model than the corresponding traits in males. In those other islands, the lack of inter-populational homogenization through migration in males represented the underlying cause favoring gender-related over variable-specific differences. Namely, due to the patriarchy that was present on most of the islands, males had to stay on their land in order to preserve it, while females migrated among villages when married. The results from Hvar island imply that this was generally not the case. However, in other island populations that were studied, this model proved considerably less applicable, probably due to specific geographic location of the villages which did not follow the straight line. Therefore, the predominance of determination of population variation by gender differences rather than variable-specific differences in those populations should be considered an artifact due to local cultural peculiarities and unsuitable geographic setting to enable applicability of the Malecot's model.

In discussion of variable-specific differences, which were very prominent in this study, it should be stated that some of the traits (e.g. monogenically determined traits) appear to show much greater rate of resistance to environmental influences known as »selective inertia«[24,39,40,80]. There is a biologically plausible explanation to this observation. Unlike the complex traits that are polygenic (have considerable additive genetic component) and highly adaptable to environmental changes, this is not the case with the inert traits. Monogenic traits lack such plasticity, their genetic architecture is based on common and ancient variants, and they have preserved the traces of ancient founding population characteristics. Thus, their rate of change under environmental influences is much smaller.

Parameter a obtained for migration kinship in Hvar population amounted to 0.01 to 0.25. That value is much higher than was observed on the other islands (in Korčula and peninsula of Pelješac they ranged from 0.0012 to 0.0019; in Brač they amounted to 0.0071 in males and 0.0171 in females)[6,9,17–21,81–83]. Relethford[59] believes that parameter a has its biological interpretation, i.e. that it represents the average unweighted »a priori« kinship, which is exactly the definition of Wright's F_{ST}[79]. Therefore, the greater values of a found on Hvar imply a

higher degree of genetic variation among the villages of Hvar. This is fully in accordance with our aforementioned thesis, that inter-population variability depends on the rate of population homogenization through migration (especially those of short-range, which are believed to be well-represented by *a* value). A basis for this can also be found in the island's specific population history. Similar *a* values were reported for other isolated populations as well, e.g., 0.0069 in Swiss alpine isolates[84], 0.0084 in Orissa, India[85] and 0.0096 on Sardinia, Italy[86].

Parameter *b* obtained for migrational kinship in Hvar population over the four periods ranged between 0.03 and 0.11. Generally, lower values of this parameter underscore the importance of long-range migrations. Higher values of *b* show a steep increase in the migrational distance coefficient with geographic distance, and therefore rapid disappearance of the isolation by distance effect[83]. It is, therefore, very interesting to note that the values of *b* for migrational kinship on the islands of Hvar, Korčula, Brač and peninsula of Pelješac ranged from 0.053 (Korčula) to 0.225 (Pelješac)[6,9,17–21,81,82]. That finding leads us to the conclusion that long-range migration characteristics were very similar for all of the islands and the

TABLE 4
PART OF CORRELATION MATRIX BETWEEN ANALYZED TRAITS CALCULATED BY MANTEL'S TEST OF MATRIX CORRESPONDENCE (ONLY VALUES GREATER THAN 0.4 WERE INCLUDED)

	KM	KINP-ISON	KINP 1	GEND-ISO	GEND-CLASS	GEND-HLA	BODY D (M)	BODY D (F)	HEAD D (M)	HEAD D (F)
KM										
KINP-ISON	–0.77									
KINP 1		0.83								
KINP 2	–0.77	0.46	0.67							
KINP 3	–0.82									
KINP 4	–0.45									
GEND-ISO										
GEND-CLASS										
GEND-HLA	0.55	–0.71	–0.55							
GEND-STR	0.41					0.47				
DEMD					0.41					
LINGD-BAS				0.75						
LINGD-CUL	0.51			0.64						
BODYD (M)	0.90	–0.85	–0.73		0.48	0.70				
BODYD (F)	0.77	–0.90	–0.76			0.81	0.94			
HEADD (M)	0.62	–0.85	–0.79			0.84	0.82	0.96		
HEADD (F)	0.68	–0.79	–0.71			0.91	0.83	0.91	0.94	
PHYSD (M)										
PHYSD (F)		–0.60						0.65	0.66	
BONED (M)				0.85						
BONED (F)	0.45	–0.52	–0.60			0.82		0.70	0.83	0.89
DERMD-QN(M)	0.51	–0.84	–0.65		0.47		0.76	0.88	0.86	0.72
DERMD-QN(F)										

peninsula in the Eastern Adriatic region, but that consequent short-range migrations within the islands/peninsula considerably varied due to specific local population history.

Model-free approach

The model-free approach included principal component analysis followed by the oblimin rotation. Relethford and Lees[59] distinguished two groups of studies of population structure which used model-free methods in order to assess quantitative variation among populations: differentiation studies and comparative studies. The former group of studies aims to determine the extent of variation among groups rather than the pattern; the latter group seeks to determine the pattern of that variation and relate it to »*...other biological, demographic, and/or historical patterns*«[59]. In this study, the pattern of correlation between measures of migrational kinship, linguistic, biological, genetic and geographic distances was assessed through the use of Mantel's test of matrix correspondence[75]. This was followed by subsequent factor analysis over correlation matrix of all distance matrices obtained on the same sample of examinees (Table 1), and the results were discussed in relation to the island's demography and population history. That means that a comparative type of model-free approach was performed in the analysis of the population structure of

TABLE 5
FACTOR LOADINGS >0.5 (OBLIMIN ROTATION)

Traits	Com.	I	II	III	IV
KM-DIST	0.86	0.50	–0.54		
KINP-ISON	0.78				0.89
KINP 1	0.32				
KINP 2	0.74		0.69		
KINP 3	0.73		0.76		
KINP 4	0.70	–0.56	0.58		
GEND-ISO	0.73			0.69	
GEND-CLASS	0.39		0.53		
GEND-HLA	0.63			0.64	
DEMD	0.76	0.87			
LINGD-BAS	0.86		–0.81		
LINGD-CUL	0.89		–0.86		
BODYD (M)	0.89	0.90			
BODYD (F)	0.93	0.96			
HEADD (M)	0.90	0.95			
HEADD (F)	0.91	0.94			
BONED (M)	0.63			0.76	–0.50
BONED (F)	0.59				0.60
PHYSD (M)	0.61			–0.57	
PHYSD (F)	0.60	0.75			
Eigenvalue	——	5.95	3.42	1.87	1.28
% Variance	——	35.0	20.0	11.0	7.6
Cumul.% V.	——	35.0	55.1	66.1	73.6

the island of Hvar. The results of initial correlation matrix of distance matrices and of subsequent direct oblimin factor rotation with $\delta = -1$ are presented, along with the eigenvalues and percentage of explained variance obtained through PCA, in Tables 4 and 5. Unrotated factors (i.e. principal components) obtained by PCA may be desirable when one is concerned with the correlations between variables, but rotated factors seem to be superior in picking out clusters of related variables[84], which is exactly the intention of our analysis. Jantz and Owsley[84] stressed that there is often a consistency of factors (or, as they call it, »*sets of complementary relationships*«) across different samples/studied populations. This, in their opinion, might be attributed to the action of genes or gene complexes, although the extent of such an effect is unclear. They stated that, if factors really can be interpreted as »*...accurate descriptions of the actions of genes or gene complexes, one would expect population differences to occur along the lines defined by factors.*«[84]. That is the thesis that we aim to delineate and discuss.

Table 4 summarizes some significant associations between 23 distance matrices (22 sets of different traits and one of geographic distances between the island's villages) calculated by Mantel's test of matrix correspondence. Patterns of the observed correlation will be briefly discussed. One can note the clusters of correlation between geographic distances and kinship estimates; further, between male and female anthropometric traits and geographic and genetic HLA distance; and finally, demographic distance with genetic distances determined from classical antigen systems.

The analysis of rotated principal components (Table 5) suggests that there is a clear separation of biological and sociocultural variables into two main groups, loading on first two factors and explaining 55.1% of total system variance. At the same time, the geographic distance, which in earlier discussion proved to be the main determinant of population structure, loaded on both aforementioned factors. One should conclude that distance measures from both groups of variables are influenced by geographic distance, but in different ways, since geographic distance loads on both components with approximately the same loading, accounting for a relatively high correlation between rotated components. All those findings indicate that the geographic distance could just partly be a determinant of population structure, and one could rightly conclude that the main source of variation within the island population in all four extracted factors (explaining 73.6% of total variance) is variable-specific.

The first component contains geographic distance, kinship for the last investigated period (the second World War and later), demographic distance, anthropometric body and head measures for both sexes, and female physiological properties, explaining 35% of total variance. *The second component* contains geographic distance, migrational kinship for three investigated periods (from 1892 until 1960, i.e. the period of extreme depopulation of the island due to economic emigration), genetic distance determined from classical antigens, and linguistic distances based on both basic and cultural vocabulary. It accounts for 20.0% of total variance. The remaining two components also contain biological traits: *the third components* contains genetic distances determined from isonymy and HLA and radiogrammetric bone distances and physiological distances in males; *the fourth component* contains kinship determined from isonymy along with radiogrammetric bone distances in both sexes. Those factors account for 11.0% and 7.6% of total variance, respectively.

The extracted components may be regarded as four »*sets of complementary relationships*«, according to Jantz and Owsley[84]. These were already observed in our studies of population structure of, respectively, the eastern Adriatic in general[85], Pelješac peninsula[5], and of the islands of Brač and Pag[6,7]. This parallelism seem to support the thesis of Jantz and Owsley[84] regarding the occurrence of population differences along the lines defined by factors, but further research on other eastern Adriatic populations is needed for comparison. Still, the results obtained so far lead us to hypothesize that factor analysis provides an insight into various levels of structuring changes along with differences in the rate of homogenization for various sets of variables. Unfortunately, it is not always possible to provide a worthy explanation to all the findings obtained by such analysis of population structure. Relethford and Lees[59] pointed out – analyzing the correlation of different distance measures – that the interpretation of the results »*...must be made with reference to all factors influencing matrix similarity...*«, and that other factors, such as degree of environmental heterogeneity among populations, and differential change over time (one can presume selective inertia of some biological and/or sex-related traits) might affect distance matrix correlation. They argued that »*...some effects are assumed to be common to a number of different distance measures...*«, while »*... other sources of variation are sexual and temporal, both in terms of developmental changes and recent migration.*«[59]. Such consistencies may represent the traces of the phenotypic expression of different genes or gene complexes, for distinguishing such common factors could be »*...the first step in the identification of individual sets of polygenes by the distribution of their effects...*«, as stated by Roberts and Coope[86].

Summary of concomitant inbreeding studies in Croatian island isolates

As an additional insight into genetic architecture of underlying variation in measured quantitative and qualitative traits, inbreeding effects on a number of quantitative and qualitative traits of both early and late onset were performed. These ranged from analyses of inbreeding effects on cancer incidence[87–89], blood pressure and anthropometric measurements[3,90,91], a number of complex chronic diseases of late onset[68,92], cognitive dysfunction[93] and oro-dental health status[94,95].

All of those studies revealed significant effects of inbreeding on the measured traits. Some effects were dramatic (e.g. on many late onset diseases and blood pressure), while some were mild (coronary heart disease) or absent (e.g. diabetes type II). The latter was only true for diseases thought to be mainly determined by environmental effects. The implications of those findings were discussed in detail[2], and they mainly suggest that the genetic architecture underlying most of the studied traits is highly complex and probably determined by a very large number of recessive variants of small individual effects. It is also possible that a smaller number of variants that are common and a few rare variants of large effect also contribute to observed variation[2,3,68].

Conclusion

In this study, the wide spectrum of traits was examined in an isolated population with presumably reduced genetic and environmental variance and using two different approaches. There was a great variability within the set of traits in compliance to the model and in revealed patterns of correlation. Some of the examined traits were more likely to be connected to specific and different selective pressures (e.g. through natural selec-

tion), others to reveal selective inertia, while for the third group of traits a reasonable interpretation of sources of variation could not always be provided[96].

The implications that these findings have for the wider context could be summarized as follows:

– The observed variation in measured human traits is a result of complex interaction between underlying genetic architecture of the traits, evolutionary forces (selective pressures) that shaped this variation in population, and a summary of extrinsic effects and their interactions with the underlying hereditary factors. We have to respect the fact that the nature and extent of any of those forces is not known with any precision to date.

– The importance of determinants of observed variation in human traits may strongly differ in different populations, so that in some (such as Hvar island) a plausible model (such as isolation by distance) could be very helpful in explaining large proportion of variation in many traits. However, the same model could prove completely useless in other populations, even if they are similar in many respects, as has been shown in some neighboring islands (e.g. Brač, Pag).

– Apart from the sources of complexity mentioned above, which are due mainly to geographic, demographic and cultural factors, this study implies that some human traits may be more favorable for studying the determinants of their hereditary variation than the others. This is reflected in differences in applicability of isolation by distance model to population structure assessed from various biological traits. The question that we need to pose is whether we could, based on a totality of observations presented, suggest any general guidelines for choosing the most promising traits for studies of determinants of their genetic variation. We believe that, when attempting these studies in the future, a care should be taken that traits of choice are:

(i) biomedically relevant (i.e., represent well-established disease risk factors);

(ii) there is evidence of their increased heritability in the isolate population of choice in comparison to general population, and possibly even some evidence of major gene(s) operating[97–100].

(iii) genetic architecture of those traits is perhaps less polygenic, and allelic heterogeneity in the population of choice is decreased[2,3];

(iv) the repeatability of standard measurements of the traits of choice is good, and there is little variation between measurements;

(v) there are no (or very few) known environmental factors influencing individual trait values, which can be controlled through public health intervention.

It is apparent that conducting studies into genetics of complex quantitative traits in an isolate population such as Hvar island has many of those points in its favor. As environmental conditions, as well as allele frequencies (and, consecutively, gene-gene and gene-environment interactions) are not uniformly shared in all human populations, some variation resulting from a particular gene pool and environmental conditions must be expected across populations, making some populations more suitable for the research than the others. In the Hvar island, there is a good medical coverage of the population considering this being an isolate, and long-term follow-ups are more likely to be possible than in outbred populations due to much lower migration rates. Heritability is expected to increase for many traits in an isolate population, mainly due to more uniform environmental ef-

fects than in large outbred communities. Finally, genetic diversity is expected to be lower in an isolate population, which should make it easier to identify genes underlying complex traits.

The analysis of this large and rather unique collection of data using both model-bound and model-free approaches through the holistic analysis showed how this extensive undertaking in an extremely isolated community still fails to explain a considerable proportion of human phenotypic variation and fails to follow model predictions in many measured traits. Therefore, the complexity of determination of human biological variation should never be underestimated.

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P. Rudan

Institute for Anthropological Research, Amruševa 8, 10000 Zagreb, Croatia
e-mail: pavao.rudan@inantro.hr

HOLISTIČKI PRISTUP ANTROPOLOŠKIM ISTRAŽIVANJIMA POPULACIJE OTOKA HVARA – OD ANALIZA RODOSLOVLJA DO ISTRAŽIVANJA DNK TIJEKOM 33 GODINE

S A Ž E T A K

Kompleksnost koja odlikuje međudjelovanja nasljednih, okolišnih i kulturoloških čimbenika u određivanju ljudskog fenotipa često se podcjenjuje u biomedicinskim istraživanjima. U ovom radu, prikazujemo rezultate 33-godišnjih holističkih antropoloških istraživanja koja se provode od 1972. godine na otoku Hvaru. Tijekom tog razdoblja, izvršen je temeljit uvid u obilježja migracija, demografije, izonimije, jezičnih različitosti, antropometrijskih svojstava, fizioloških (kardiorespiratornih) svojstava, kvantitativnih i kvalitativnih dermatoglifskih svojstava, radiogramskih obilježja metakarpalnih kostiju i genetskih svojstava (sustava eritrocitnih antigena, HLA, DNK kratkih ponavljajućih sljedova, te polimorfizama mitohondrijske DNK i Y-kromosoma). Analiza ove velike kolekcije podataka primjenom modela i bez primjene modela pokazala je da bi kompleksnost u podlozi bioloških svojstava mogla biti i znatno veća no što se općenito pretpostavlja, što ima važne posljedice za dizajniranje budućih istraživanja nasljednih odrednica kompleksnih svojstava.