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Molecular Characterization and Genetic Diversity Analysis of Sweet Orange (*Citrus sinensis* L. Osbeck) Cultivars in Iraq Using RAPD Markers

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Abstract

Sweet orange (*Citrus sinensis* L. Osbeck) is one of the most important commercially cultivated fruit crops of Citrus. Genetic diversity and inter-relationship among 5 cultivars (Indian, Iraqi, Japanese, Syrian, Egyptian) of *C. sinensis* were analyzed based on RAPD markers. Six primers generated reproducible and easily storable RAPD profiles with a number of amplified DNA fragments ranging from 6 to 14 fragment bands. The total number of amplicons detected was 51, including 14 fragments unique bands with average reached 2.8 fragments / primers. While the number of polymorphic ranged from 0 to 8 with an average reached 4.4 fragments / primers with the polymorphic percentage ranged from 0% to 57.1%. While the number of monomorphic ranged from 2 to 5 fragment bands and was total of the monomorphic 15 fragments with an average reached 3 fragments / primers with the monomorphic percentage was 14.2 % to 83.3%. A maximum numbers of amplicons was amplified with primer OPS-238 reached 14 fragments while the minimum number of fragments was amplified with primer OPS-253 reached 6 fragments. The highest number of polymorphic bands reached 8 fragments was obtained with primer OPS-238 with high percentage 57.1%, while the highest number of monomorphic bands reached 5 fragments with high percentage 83.3% was obtained with primer OPS-253. RAPD markers detected genetic distance and similarity, amaximum genetic distance value was observed between Japanese (Jap) and Syrian (Syr) cultivars reached 0.530 with less similarity value reached 47%, a minimum genetic distance value was observed between sweet Iraqi (Irq) and Indian (Ind) cultivars reached 0.239 with high similarity value reached 76.1%. The similarity matrices were employed in the cluster analysis to generate a dendrogram using the UPGMA method. The cluster tree analysis showed that the sweet orange cultivars were broadly divided into two main groups A and B with similarity reached 50%. A group including individual one cultivars was Japanese. B group was divided into two sub-cluster B1 and B2 with genetic similarity reached 63%. The first sub-cluster (B1) was included two cultivars Iraqi and Indian with high genetic similarity among other cultivars reached 77%. The second sub-cluster (B2) was included two cultivars Egyptian and Syrian with genetic similarity reached 72%.

Keywords: sweet orange, genetic diversity, molecular marker, RAPD.

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1. Introduction

Citrus is one of the most economically important fruit crops of the world, belonging to the subfamily Aurantioideae of the family Rutaceae. It is widely distributed throughout the tropical and subtropical regions of the world and believed to have originated in Southeast Asia, particularly northeast India, the Malayan archipelago, China, Japan, and Australia [1, 2]. Among the cultivated species (*Citrus sinensis* L. Osbeck) sweet orange is the most important commercial fruit crop of Citrus and believed to be a hybrid between pummelo (*Citrus maxima*) and mandarin (*Citrus reticulata*) [3, 4]. It is a highly poly embryonic species, fruit pulp is used for preparing fresh juice which is rich in vitamin C and protein content and peel of the fruit is used for making perfume and soaps. Cooking oil is extracted from its seeds, Juice extracted from its leaves is used to control several diseases like ulcers, sores, etc. [5]. The use of molecular markers has been a valuable and precise strategy to identify Citrus species, cultivars and biotypes and to investigate the genetic diversity of Citrus species. Molecular marker techniques like RAPD, ISSR, RFLP, SSR, AFLP and other markers have been used for germplasm characterization, studies of genetic diversity, systematics and phylogenetic analysis [6]. Among them, random amplified polymorphic DNA (RAPD) markers have been employed most widely for characterization of plant species [7]. Most citrus species genera are diploid (2n = 2x = 18), with relatively small genomes; for instance, sweet orange (*Citrus sinensis* L. Osbeck) has a genome of about 367 Mb, nearly three times the size of the 125 Mb Arabidopsis genome [8, 9]. The conventional methods in *Citrus* cultivars identification relied on morphological features and isozymes [10]. In the cultivated citrus, sweet orange (*C. sinensis* L. Osbeck) originated as a natural hybrid between mandarin and pummel, showed low level of genetic diversity according to lots of previous studies [11-14]. The authors [15] suggested that sweet orange has a majority of its genetic makeup from mandarin and only a small proportion from pummelo. Grapefruit was reported as a hybrid of pummelo and sweet orange [16, 4, 17], and all grapefruit cultivars originated from single parent through mutations [18, 19]. It is notified that most of sweet oranges obtained by mutation from one ancestor tree, So despite of differences in morphological characters, genetic variation of sweet orange was low [20]. Using morphological traits, it is difficult to distinguish between many *Citrus* cultivars because some cultivars are distinguishable only by fruit traits and *Citrus* trees usually do not bear fruits until 3-4 years after planting. Moreover, isozyme markers can be mediated by secondary processes so that the normal patterns of expression are suppressed. Phenotypic diversity, poly embryonic, hybridization and mutations have prevented consensus on systematic classification of *Citrus* [21] and hampered *Citrus* improvement programs. The development of molecular markers based on DNA sequences has provided an ideal means for identifying genotypes, estimation of relatedness between different accessions and following inheritance of economically important characters, a wide variety of DNA-based markers have been developed in the past few years, [22]. Orange cultivars are classified into four groups: common, low acidity, pigmented and navel oranges [23 as cited in 13]. It is indicated despite the existence of substantial diversity among cultivated genotypes in respect of morphological, physiological and agronomic traits, very little DNA variation has been detected using DNA markers [13]. In Citrus, RAPD markers have been used for cultivar identification, genetic mapping, genetic diversity assessment and other breeding programs and RAPD marker have gained more attention due to the simplicity of the procedure, the low cost and the very small amount of the DNA required for analysis [12, 21, 24-29]. They notified that sweet oranges have a narrow genetic basis and that most morphological characters originated through mutations, and clonal propagation of sweet oranges is the case for the majority of citrus species [30, 14]. In paper [20] were used ISSR markers to differentiate 41 samples of orange belongs to three groups. This notified as majority of sweet orange cultivars derived from a single ancestor by mutation. However, some cultivar distinguished from others. In other study, it was found identical microsatellite profiles at 9 out of 10 SSR loci among analyzed orange cultivars and clones [31]. RAPDs have been extensively used in assessing relationships amongst various accessions of different plant species [32-35].

The objectives of the present study are to determine the genetic variability among 5 *Citrus sinensis* L. (Sweet orange) genotypes using RAPD markers and to assess the genetic relationships among these genotypes. Addition to knowledge of genetic variation and genetic relationship among genotypes is an important consideration for classification, utilization of germplasm resources and breeding.

2. Materials and methods

Plant material

A total of 5 sweet orange (*Citrus sinensis* L.) cultivars genotypes (Indian, Iraqi, Japanese, Syrian, Egyptian) used in this study were collected from the citrus private orchard of Babylon-Iraq.

DNA isolation

The total genomic DNA for 5 sweet orange was isolated from fully expanded leaves using the Kit. Leaf samples (300 mg) were ground to a fine powder in liquid nitrogen. DNA was extracted by using Genomic DNA Mini Kit (Geneaid, UK). The extracted DNA (200 µl) was stored at –20 °C until use. Concentration, quality and quantity of the DNA were determined by Nano drop-spectrophotometrically at λ = 260 nm. Stock DNA samples were stored at –20 °C and diluted to 20 ng uL^{-1} when in use. The analysis was conducted in the laboratory of Molecular Genetics at the university of Baghdad, genetic engineering and biotechnology institution.

PCR procedure

The RAPD primers (Table 1) were purchased from BIONEER, South Korea. A total of 6 decamer oligonucleotides of arbitrary sequence were tested for PCR amplification. AccupPower Gold Multiplex PCR premix (BIONEER, South Korea) was used to the DNA amplification with RAPD primers. The PCR were carried out in 25 µl reactions. The temperature profile for the reaction is given as: hot start at 95 °C (only at the start of reaction) for 5 min., denaturation at 95 °C for 1 min., primer annealing at 36 °C for 1 min., extension at 72 °C for 2 min. and final extension at 72 °C (only at the end of reaction) for 10 min.

DNA electrophoresis

Amplification products were separated by electrophoresis (100v for 30 min.) in 1.5 % agarose gels (1.5 mg mixed with 80 ml of TBE buffer) and stained in ethidium bromide. A photographic record was taken under the UV illumination document gel.

Data analysis

Only clear and repeatable application products were scored as 1 for present bands and 0 for absent ones. The specific bands useful for identifying species and cultivar were named with primer number followed by the approximate size of the amplified fragment in base pairs. Polymorphism was calculated based on the presence or absence of bands. Molecular weight of the amplified bands was estimated by using a 1 Kb DNA ladder (BIONEER, South Korea). Amplified products were analyzed by pairwise comparisons of the genotypes based on the percentage of common fragments, and a similarity matrix was calculated [36]. The 0 or 1data matrix was created and used to calculate the genetic distance and similarity using 'Simqual', a subprogram of the NTSYS-PC program (numerical taxonomy and multivariate analysis system program) [37]. A dendrogram was constructed based on the genetic distance matrix by applying an unweighted pair group method with arithmetic averages (UPGMA) cluster analysis using the MEGA (Molecular Evolutionary Genetics Analysis) version 2.0 [38].

3. Results and discussion

Figure 1 shows the results of the isolated total DNA of the leaves of the studied sweet orange cultivars manner filters and then migrated to agar gel 1.5%, electric voltage 100 V for 30 min. noting the success of the method to isolate the DNA from these sweet orange cultivars.

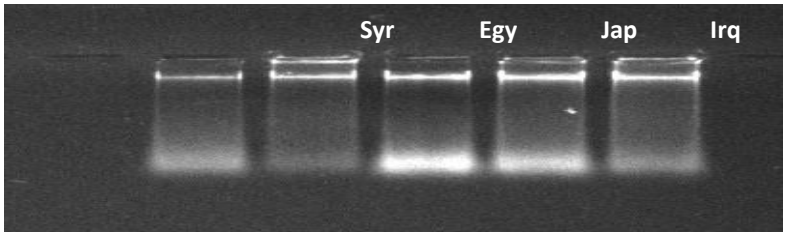

Figure 1. the isolated total DNA of the Sweet orange *C. sinensis* cultivars leaves Ind: Indian, Irq: Iraqi, Jap: Japanese, Egy: Egyptian, Syr: Syrian on agarose gel (1.5%) and electric voltage (100 V) for (30 min.)

Polymorphisms and monomorphisms detected by RAPAD markers:

One of the most important features of the RAPD technique is detecting of high levels of polymorphism and this feature has been met in present study (Fig. 2). Six primers were screened with the DNA of the 5 sweet orange cultivars genotypes. All 6 primers tested were generated reproducible and showed easily storable RAPD profiles with a number of amplified DNA fragments ranging from 6 to 14 (Table 1).

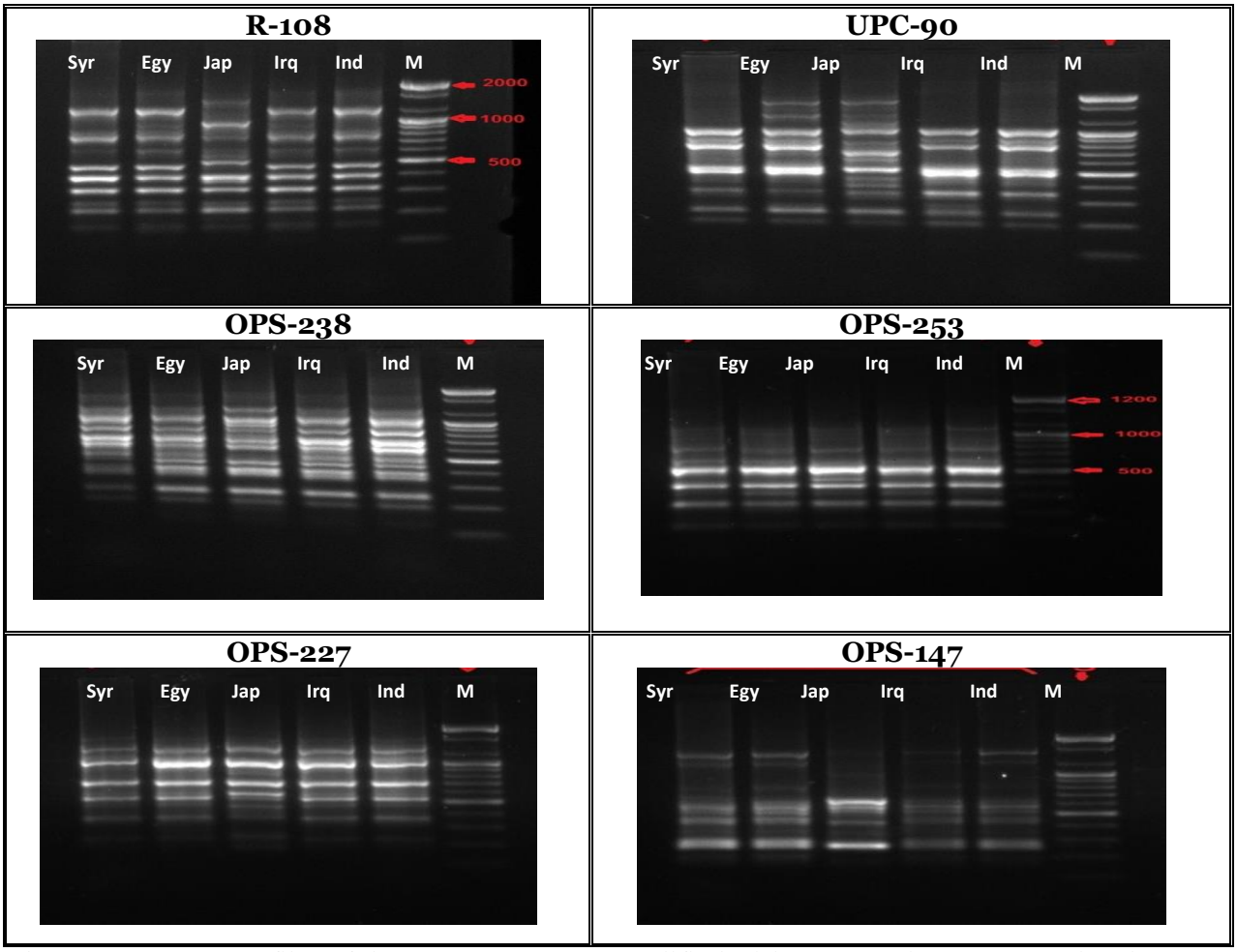

Figure 2. RAPD profiles of the 5 sweet orange amplified with RAPD primers, M: molecular weight marker, Culvers Ind: Indian, Irq: Iraqi, Jap: Japanese, Egy: Egyptian, Syr: Syrian on agarose gel (1.5%) and electric voltage (100 V) for (30 min.)

The total number of fragments produced by 5 primers was 51 with an average of 10.2 fragments / primers. The high unique fragment appeared in OPS-238 and UPC-90 primers reached 4 with high percentage of 28.5% and 30.2% respectively. While the number of polymorphic ranged from 0 to 8 with an average reached 4.4 fragments / primers with the polymorphic percentage ranged from 0% to 57.1%. While the number of monomorphic ranged

from 2 to 5 and was total of the monomorphic 15 with an average reached 3 fragments / primers with the monomorphic percentage was 14.2% to 83.3%. As shown in Table 1 a maximum numbers of amplicons was amplified with primer OPS-238 reached 14 while the minimum number of fragments was amplified with primer OPS-253 reached 6. The highest number of polymorphic bands reached 8 was obtained with primer OPS-238 with high percentage 57.1%, while the highest number of monomorphic bands reached 5 with high percentage 83.3% was obtained with primer OPS-253.

Table 1. Total number and percentage of amplicons, Unique, polymorphic, monomorphic amplicons as revealed by RAPD markers among the 5 sweet orange cultevars accessions

RAPD Primers	Primer sequences 5' to 3'	Number of Total amplified fragments	Number of Unique Fragments bands	Unique Fragments Bands Percentage (%)	Number of Polymorphic Fragments Bands	Polymorphism Fragments Percentage (%)	Number of Monomorphic Fragments Bands	Monomorphic Fragments Percentage (%)
OPS-238	TGGTGG CGTT	14	4	28.5	8	57.1	2	14.2
OPS-253	GGCTGG TTCC	6	1	16.6	0	0	5	83.3
R-157	GCTGG TTCCT	9	3	33.3	4	44.4	2	22.2
R-108	GTATTG CCCT	9	2	22.2	4	44.4	3	33.3
UPC-90	GGGGGTTAGG	13	4	30.2	6	46.1	3	23.0
TOTAL		51	14		22		15	
Average		10.2	2.8	26.16	4.4	38.4	3	35.2

When compared among sweet orange cultivars shown from RAPD marker data that high fragments number were observed in Syrian cultivar reached 39 fragments band, while the less fragments number was observed in Egyptian cultivar reached 28 fragments band (Table 2).

Table 2. Sweet orange cultivars fragments numbers RAPD markers

Genotype(cultivars)	Number of total Fragment
Ind	36
Irq	38
Jap	37
Egy	35
Syr	39

Notes: Cultivars, Ind: Indian, Irq: Iraqi, Jap: Japanese, Egy: Egyptian, Syr: Syrian.

Genetic distance and relationships among sweet orange cultivars by used RAPD markers:

Table 3 showed that data of RAPD markers scanned from 5 sweet orange cultivars with reproducible primers were used to genetic distance and similarity value co-efficient. Amiximum genetic distance value was observed between Japanese and Syrian reached 0.530 with less similarity value reached 43%. While a minimum genetic distance value was observed between Iraqi and Indian reached 0.239 with high similarity value reached 76.1% (Table 3).

Table 3. Genetic distance among sweet orange cultivars

	Ind	Irq	Jap	Egy	Syr
Ind	**0.000**	**0.239**	**0.480**	**0.286**	**0.426**
Irq	**0.239**	**0.000**	**0.5**	**0.3182**	**0.417**
Jap	**0.480**	**0.5**	**0.000**	**0.480**	**0.530**
Egy	**0.286**	**0.318**	**0.480**	**0.000**	**0.280**
Syr	**0.426**	**0.417**	**0.530**	**0.280**	**0.000**

Notes: Cultivars, Ind: Indian, Irq: Iraqi, Jap: Japanese, Egy: Egyptian, Syr: Syrian

To determine the genetic relationships among 5 sweet orange cultivars, the scoring data were used to compute the similarity matrices. These genetic similarity matrices were then used in the cluster analysis to generate a dendogram using in the cluster analysis UPGMA analysis. The cluster tree analysis (Fig. 3) showed that the cultivars were broadly divided into two main groups A and B with genetic similarity reached 50%. A group including individual one cultivar was Japanese. B group was divided into two sub-cluster B1 and B2 with genetic similarity reached 63%. The first sub-cluster (B1) was included two cultivars Iraqi and Indian with high genetic similarity among other cultivars reached 77%. The second sub-cluster (B2) was included two cultivars Egyptian and Syrian with genetic similarity reached 72%.

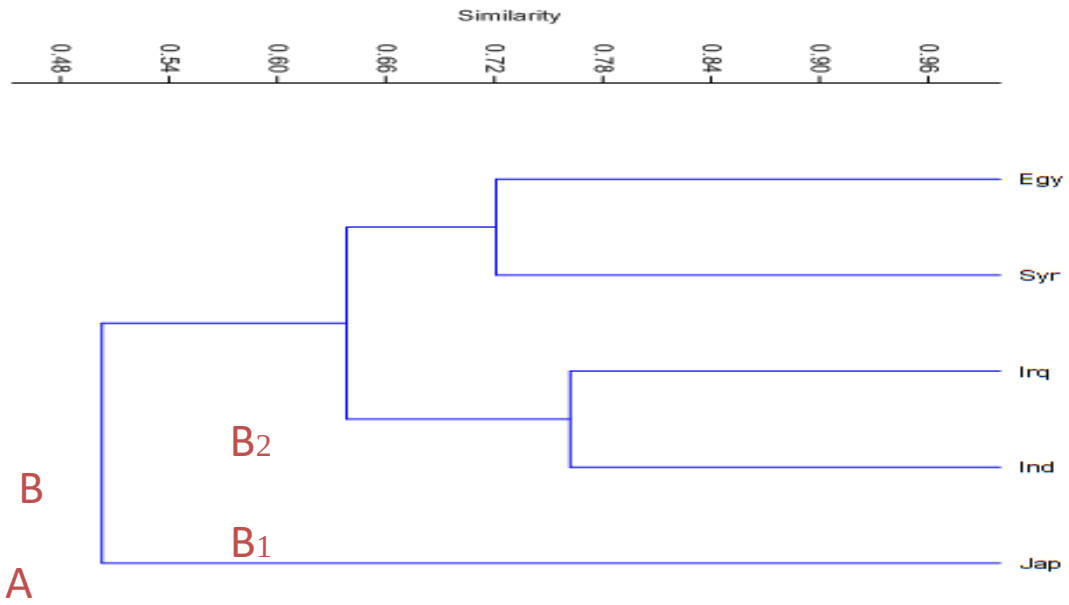

Figure 3. Dendrogram for the 5 sweet orange cultivars constructed from RAPDs data using Unweighted Pair-group Arithmetic Average (UPGMA) and similarity matrices computed according to coefficients. Cultivars, Ind: Indian, Irq: Iraqi, Jap: Japanese, Egy: Egyptian, Syr: Syrian.

The same results were also reported in other studies. The authors [16] notified variations in orange, lemon, grapefruit and lime based on mutations occurred on one ancestor tree. In paper [39] reported that it was difficult to distinguish cultivars originated mutations using isozyme markers. Low level of polymorphism in orange also found with ISSR [20], SSR [40, 13], SRAP [41]. On the other hand, no variation was found in studied oranges in some researches [42]. Orange cultivars are classified into four groups: common, low acidity, pigmented and navel oranges [23 as cited in 12]. It is indicated that despite the existence of substantial diversity among cultivated genotypes in respect of morphological, physiological and agronomic traits, very little DNA variation has been detected using DNA markers [13]. Same researchers found low level of genetic

polymorphism among 41 orange cultivars, they notified that sweet oranges have a narrow genetic basis and that most morphological characters originated through mutations, and clonal propagation of sweet oranges is the case for the majority of citrus species [30 as cited in 43]. In paper [20] were used ISSR markers to differentiate 41 samples of orange belongs to three groups, Valencia, blood and navel based on fruit traits. All of these cultivars found almost the same ISSR fingerprints. This was notified as majority of sweet orange cultivars derived from a single ancestor by mutation. However, some cultivar distinguished from others, it is explained as only a case in which replicate samples of the same cultivar from different locations had different ISSR fingerprint patterns, it is indicated that this result suggests that mutation occurred in at least one of them, although horticultural traits are not known between them [20]. In other study, it was found identical microsatellite profiles at 9 out of 10 SSR loci among analyzed orange cultivars and clones [12, 44].

4. Conclusion

In recent study carried out using large amount of orange showed that there was high level of genetic similarity in oranges [14, 41]. Similarity level of it derived from zygotic origin. Genetic similarity of all of other oranges was over 69% and some of them were identical, in this group there were many common orange cultivars and clones such as, many Japanese, Egyptian, Indian, Syrian introduced from other countries. Molecular marketing techniques may be a first step towards efficient conservation, maintenance and utilization of existing genetic diversity of sweet orange plants. This may lead further to different genetic analysis, gene mapping and ultimate improvement of the crop at genetic level. Randomly amplified polymorphic DNA (RAPD) markers are usually preferred in this kind of work as the technique is simple, versatile and relatively inexpensive and can detect minute differences [7]. The Citrus RAPD markers have been used for genetic mapping [45], identification of cultivars [21], hybrids [46], mutants [47], chimerase [48] and phylogenetic analyses [4]. Random PCR approaches are being increasingly used to generate molecular markers which are useful for taxonomy and for characterizing populations. The main advantages of these approach is that previous knowledge of DNA sequences is not required, so that any random primer can be tested to amplify any fungal DNA. RAPD primers are chosen empirically and tested experimentally to find RAPD banding patterns which are polymorphic between the isolates studied. Using PCR-RAPD, [49-51] it was also possible to identify heterogenity with in groups of genotypes which originates within the same location. In present study, characterization of sweet orange varieties by the RAPD has proved useful in separating all varieties/clones from each other. It has also provided us with primer markers that can be used to separate and distinguish each clone. In paper [15] it was suggested that sweet orange has a majority of its genetic makeup from mandarin and only a small proportion from pummelo. The demonstrated Genetic distance and Genetic relationships as revealed by RAPD markers results that nearest cultivars were Iraqi and Indian and farthest genetically were among Japanese and other cultivars. The reason is probably due to different environmental were formerly breaded on them and reflected it on genetic materials. So the environmental condition convergent may be caused semi genetic makeup (like Iraq and India).

References:

1. Moore, G.A. (2001) Oranges and lemons: clues to the taxonomy of Citrus from molecular markers. Trends Genet 17: 536–540.

2. Swingle, W.T. and Reece, P.C. (1967) The botany of Citrus and its wild relatives / In: Reuther, W., Batchelor, L.D., Webber, H.J. The citrus industry, vol. I. University of California Press, Berkeley, pp. 190–340.

3. Davies, F. and Albrigo, L.G. (1992) Taxonomy, cultivars and breeding. In: Gmiter, FG. Jr.; Grosser, J.W.; Moore G.A. (eds) Citrus. CAB International, Wallingford, pp 12–51.

4. Nicolosi, E.; Deng, Z.N.; Gentile, A. and La Malfa, S. (2000) Citrus phylogeny and genetic origin of important species as investigated by molecular markers. Theor Appl Genet 100: 1155–1166.

5. Dugo, G. and Giacomo, Di. (2002) A Citrus: The genus Citrus, medicinal and aromatic plants—industrial profiles. – Taylor and Francis, London.

6. Weising, K.; Nybom, H. Wolff, K. and Kahl, G. (2005) DNA fingerprinting in plants' principles, methods and applications, 2nd edn. Taylor and Francis, Boca Raton.

7. Williams, J.G.K.; Kublik, A.R.; Livak, K.J.; Rafalsky, J.A. and Tingey, S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acid. Res. 18: 6531-6535.

8. Arumuganathan, K. and Earle, E.D. (1991) Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep. 9: 208–218.

9. Das, A.; Sarkar, J.; Mondal, B. and Chaudhury, S. (2004) Genetic diversity analysis of Citrus cultivars and rootstocks of Northeastern India by RAPD markers. Indian J. Genet. 64: 281–285.

10. Protopapadadis, E.E. (1988) Effect of rootstocks on isoenzymic composition of Citrus. Proceeding of the Sixth International Citrus Congress. pp. 609-614.

11. Luro, F.; Laigrent, F.; Bove, J.M. and Ollitrault, P. (1995) DNA amplified fingerprinting, a useful tool for determination of genetic origin and diversity analysis in Citrus. Hort Science 30: 1063-1067.

12. Novelli, V.M.; Cristofani, M. and Machado, M.A. (2000) Evaluation of microsatellite markers in cultivars of sweet orange (Citrus sinensis (L.) Osbeck. Acta Hort 535:47–49.

13. Novelli, V.M.; Cristofani, M.; Souza, A.A. and Machado, M.A. (2006) Development and characterization of polymorphic microsatellite markers for the sweet orange (Citrus sinensis L. Osbeck). Genetics and Molecular Biology. 29: 90-96.

14. Uzun, A.; Yesiloglu, T.; Aka-Kacar, Y.; Tuzcu, O. and Gulsen, O. (2009) Genetic diversity and relationships within Citrus and related genera based on sequence related amplified polymorphism markers (SRAPs). Scientia Horticulturae, 121: 306–312.

15. Barkley, N.A.; Roose, M.L.; Krueger, R.R. and Federici, C.T. (2006). Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). Theor. Appl. Genet. 112, 1519–1531.

16. Barrett, H.C. and Rhodes, A.M. (1976) A numerical taxonomic study of affinity relationships in cultivated Citrus and its close relatives. Syst. Bot. 1: 105–136.

17. Maya, M.A.; Rabbani, M.G.; Mahboob, M.G. and Matsubara, Y. (2012) Assessment of genetic relationship among 15 Citrus fruits using RAPD. Asian J. Biotech. 4: 30–37.

18. Corazza-Nunes, M.J.; Machado, M.A.; Nunes, W.M.C.; Cristofani, M. and Targon, M.L.P.N. (2002) Assessment of genetic variability in grapefruits (Citrus paradise Macf.) and pummelos (C. maxima Burm. Merr.) using RAPD and SSR markers. Euphytica 126: 169–176.

19. Abkenar, A.A.; Isshiki, S. and Matsumoto, R. (2007) Comparative analysis of organelle DNAs acid citrus grown in Japan using PCR-RFLP method. Genet. Res. Crop. Evol. 55: 487–492.

20. Fang, D.Q. and Roose, M.L. (1997) Identification of closely related Citrus cultivars with inter simple sequence repeat markers. Theor. Appl. Genet. 95: 408–417.

21. Coletta, F.H.D.; Machado, M.A.; Targon, M.L.P.; Moreira, M.C.P. and Pompeu, J. (1998) Analysis of the genetic diversity among mandarins (Citrus spp.) using RAPD markers. Euphytica 102: 133-139.

22. Botstein, D.; White, R.L.; Skolnick, M. and Davis, R.W. (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Amer. J. Genet. 32: 31.

23. Hodgson, R.W. (1967) Horticultural varieties of citrus. In: Reuther, W., Webber, H.J., Batchelor, L.D. (Eds.). The Citrus Industry, vol. 1. University of California Press, Berkeley, pp. 431–591.

24. Luro, F.; Laigrent, F.; Bove, J.M. and Ollitrault, P. (1992) Application of random amplified polymorphic DNA (RAPD) to Citrus genetics taxonomy. Proc Int. Citriculture 1: 225–228.

25. Cai, Q.; Guy, C.L. and Moore, G.A. (1994) Extension of the linkage map in Citrus using random amplified polymorphic DNA (RAPD) markers and RFLP mapping of cold-acclimation-responsive loci. Theor Appl Genet 89:604–614.

26. Cheng, F.S. and Roose, M.L. (1995) Origin and inheritance of dwarfing by the Citrus rootstock Poncirustrifoliata'Flying Dragon'. J. Amer. Soc. Hort. Sci. 120: 286- 291.

27. Natividade, A.; Targon, M.L.P.; Machado, M.A.; Coletta Filho, H.D. And Cristofani, M. (2000) Genetic polymorphism of sweet orange (Citrus sinensis L. Osbeck) varieties evaluated by random amplified polymorphic DNA. Acta. Hort 535: 51–53.

28. Bidisha, M.; Reeya, D. and Saha, R. (2013) Application of DNA Based Molecular Marker for the Assessment of Genetic Transformation in Citrus Sinensis. International Journal of Science and Research (IJSR), Index (6): 2319-7064.

29. Abkenar, A. and Isshiki, S. (2003) Molecular characterization and genetic diversity among Japanese acid Citrus (*Citrus spp.*) based on RAPD markers. J. Hort. Sci. Biotech. 78: 553–556.

30. Herrero, R.; Asins, M.J.; Carbonell, A.E. and Navarro, L. (1996) Genetic diversity in the orange subfamily Aurantioideae. I. Intraspecies and intragenus genetic variability. Theor. Appl. Genet. 92: 599–609.

31. Hvarleva, T.; Kapari-Isaia, T.; Papayiannis, L.; Atanassov, A.; Hadjinicoli, A. and Kyriakou, A. (2008) Characterization of citrus cultivars and clones in Cyprus through microsatellite and RAPD analysis. Biotechnol. And Biotechnol. Eq. 22: 787-794.

32. Ahmed, F. (1999) Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among annual Cicerspecies. Theor. Appl. Genet. 98: 657-663.

33. Nebauer, S.G.; Gastillo-Agudo, L. and Segura, J. (2000) An assessment of genetic relationships within the genus Digitalis based on PCR-generated RAPD markers. Theor. Appl. Genet. 100: 1209-1016.

34. Besnard, G.; Baradat, P. and Bervill, A. (2001) Genetic relationships in the olive (Olea europaea L.) reflect multilocal selection of cultivars. Theor. Appl. Genet. 102: 251-258.

35. Iruela, M.; Rubio, J.; Cubero, J.I.; Gil, J. and Millan, T. (2002) Phylogenetic analysis in the genus Cicerand cultivated chickpea using RAPD and ISSR markers. Theor. Appl. Genet. 104: 643-651.

36. Nei, M. and Li, W.H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceed of the National Academy of Sciences of the USA, 76, 5269-5273.

37. Rohlf, F.J. (2005) NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System Version 2.1. Exeter Publications, Setauket, New York, 2: 02.

38. Kumar, S., Tamura, K., Jakobsen, I. B. and Nei, M. (2001). MEGA 2: Molecular Evolutionary Genetics Analysis software, Bioinformatics, 17, 1244-1245.

39. Roose M.L. (1988) Isozymes and DNA restriction fragment length polymorphisms in Citrus breeding and systematics. Proc. Int. Soc. Citricult. VI. Congr. Margraf Scientific Books, Weikersheim, pp. 155–165.

40. Luro, F.; Rist, D. & Ollitrault, P. (2000) Sequence tagged microsatellites polymorphism: an alternative tool for cultivar identification and evaluation of genetic relationships in Citrus. Proc. Int. Soc. Citricult. IX. Congr. Florida, USA. Pp. 170–171.

41. Uzun, A. (2009) Characterization of genetic diversity of Citrus by SRAP markers. Ph.D. Thesis. University of Cukurova, Institute of Natural and Applied Sciencies. p. 369.

42. Qing-Qin, C.; Hai-Jun, M.; Xiao-Peng, W.; Hua-Lin, Y. & Xiu-Xin, D. (2007) Genetic diversity of male sterile and low fertility germplasm of Citrus revealed using SSR markers. Chinese Journal of Agricultural Biotechnology 4: 99–104.

43. Breto, M.P.; Ruiz, C.; Pina, J.A. & Asins, M.J. (2001) The diversification of Citrus clementina Hort. Ex Tan., a vegetative propagated crop species. Mol. Phylo. Evol. 21: 285-293.

44. Sankar, A.A. and Moore, G.A. (2001) Evaluation of inter-simple sequence repeat analysis for mapping in Citrus and extension of the genetic linkage map. Theor Appl Genet 102: 206–214.

45. Cristofani, M.; Figueiredo, J.O.; Targon, M.L.P.N and Machado, M.A. (2003) Differentiation of lemon varieties by microsatellites. Journal of Laranja. 24(1): 165-175.

46. Elisiario, P.J.; Justo, E.M. and Leitao, J.M. (1999) Identification of Mandarin hybrids by isozyme and RAPD analysis. Scientia Horticulture. 81: 287-299.

47. Deng, Z.N.; Gentile, A.; Nicolosi, E.; Domina, F.; Vardi, A. and Tribulato, E. (1995) Identification of in vivo and in vitro lemon mutants by RAPD markers. J. Hort. Sci. 70(1): 117-125.

48. Sugawara, M.K.; Oowada, A., Moriguchi, T. and Omura, M. (1995) Identification of citrus chimeras by RAPD markers. Journal of Hortscience. 30(6): 1276- 1278.

49. Duncan, S.; Barton, J.E and Brien, P.A.O. (1993) Analysis of variation in isolates of Rhizoctoniasolaniby random amplified polymorphic DNA assay. Mycological Research. 1075-1082.

50. Deng, Z.; Huang, S.; Xiao, Y.S. and Gmitter, F.G. (1997) Development and characterization of SCAR markers linked to the Citrus tristeza virus resistance gene from Poncirustrifoliata. Genome 40: 697-704.

51. Fang, D.Q.; Federici, C.T. and Roose, M.L. (1997) Development of molecular markers linked to a gene controlling fruit acidity in Citrus. Genome 40: 841-849.

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Studying the Composition and Properties of Mountain and Melt Water of Bulgaria and Russia as Factors of Longevity. Effects of Calcium, Magnesium, Zinc and Manganese in Water on Organism

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Abstract

This paper demonstrates that mountain and melt water is among the most important factors for longevity. Other factors are hereditary, gender, body weight, food, psychological status, family relationships. Natural waters derived from various Bulgarian water springs, as well as melt water and blood serum of cancer patients between 50 and 70 years old were investigated by IR, NES and DNES-methods. We applied the NES- and DNES-methods for calculation of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules in the samples, as well as the percent distribution of H_2O molecules according to energies of hydrogen bonds within intervals (from -0.08 to -0.1387 eV). As estimation factor was measured the values of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules, as well as local extremums in DNES- and IR-spectra of various samples of water and human blood serum, detected at E = -0.1387 eV and λ = 8.95 μm. For a group of people in critical condition of life and patients with malignant tumors the greatest values of local extremums in DNES-spectra are shifted to lower energies relative to the control group. The IR-spectrum of mountain water is most similar to the IR-spectrum of blood serum of healthy group of people with a local maximum at λ = 8.95 μm. Natural mountain and melt water with unique chemical composition and less deuterium content seems to be one of the most important factors for longevity. In Bulgaria, most long lived people and centenarians live in the Rhodope Mountains, while in Russia – in Dagestan and Yakutia. The similar characteristics possess mountain water from Teteven and other Bulgarian sources. There are new proofs for biophysical and biochemical effects of Ca^{2+}, Mg^{2+}, Zn^{2+} and Mn^{2+} in water.

Keywords: longevity, mountain water, melt water, IR spectroscopy, NES, DNES.

1. Introduction

Water is the main substance of life. The human body is composed from 48 to 54% of water for adult people. With aging, the percentage of water in the human body decreases. Hence, the factor of water quality is the essential factor for the research. Water is present in the composition of the physiological fluids in the body and plays an important role as an inner environment in which the vital biochemical processes involving enzymes and nutrients take place. Water is the main

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factor for metabolic processes and aging [1]. Earlier studies conducted by us have demonstrated the role of water, its structure, isotopic composition and physico-chemical (pH, temperature) in the growth and proliferation of prokaryotes and eukaryotes in water with different isotopic content [2–4]. These factors and the structure of water are of great importance in biophysical studies. The peculiarities of chemical structure of H_2O molecule create favorable conditions for formation of electrostatic intermolecular van der Waals, dipole-dipole forces and donor-acceptor interaction with transfer of charges between H-atom and O-atoms in H_2O molecules, binding them into water associates (clusters) with the general formula $(H_2O)_n$ where n varies from 3 to 50 units [5].

Other important indicator of water quality is its isotopic composition. The natural water consists on 99.7 mol.% of $H_2{}^{16}O$, which molecules are formed by ^1H and ^{16}O atoms [6]. The remaining 0.3 mol.% is represented by isotope varieties (isotopomers) of water molecules, wherein deuterium forms 6 configurations of isotopomers – $HD^{16}O$, $HD^{17}O$, $HD^{18}O$, $D_2{}^{16}O$, $D_2{}^{17}O$, $D_2{}^{18}O$, while 3 configuration are formed by isotopomers of oxygen – $H_2{}^{16}O$, $H_2{}^{17}O$, $H_2{}^{18}O$.

In frames of this research 415 people living in the municipalities of Teteven, Yablanitza. Ugarchin, Lukovit, Lovech district; Dolni Dabnik, Pleven district, Kuklen, Pleven district (Bulgaria), where is lived the most of long lived people and their siblings, were studied.

2. Material and Methods

2.1. Preparation of Water Samples with Varying Deuterium Content

For preparation of water samples with varying deuterium content we used D_2O (99.9 atom%) received from the Russian Research Centre "Isotope" (St. Petersburg, Russian Federation). Inorganic salts were preliminary crystallized in D_2O and dried in vacuum before using. D_2O distilled over $KMnO_4$ with the subsequent control of deuterium content in water by ^1H-NMR-spectroscopy on Brucker WM-250 device ("Brucker", Germany) (working frequency – 70 MHz, internal standard – Me_4Si) and on Brucker Vertex ("Brucker", Germany) IR spectrometer (a spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; the permission – 0,5 cm^{-1}; accuracy of wave number – 0,1 cm^{-1} on 2000 cm^{-1}).

2.2. Preparation of melt water

The melt water was obtained from Moscow tap water by the freeze-thaw method in a standard procedure: 1.5 l of Moscow tap water was placed in a glass jar with a lid and placed in the refrigerator freezer at -14 ^oC for 4–5 hours. Then, the first ice crystals were mechanically removed from the mixture, and the jar again was placed in the freezer additionally for 8–10 hours before ¾ of liquid freezes. Thereafter, the liquid brine is decanted and the remaining ice was thawed at room temperature and used for further experiments. The melt water was stored in a glass container in refrigerator. Other experiments were carried out with deuterium depleted water (DDW) with residual deuterium content of 60–100 ppm, purchased from Langway Water Inc. (Moscow, Russia).

2.3. DNES Spectral Analysis

The device for DNES was made by A. Antonov on an optical principle. In this study was used a hermetic camera for evaporation of water drops under stable temperature (+22–24 ^oC) conditions. The water drops are placed on a water-proof transparent pad, which consists of thin maylar folio and a glass plate. The light is monochromatic with filter for yellow color with wavelength λ = 580±7 nm. The device measures the angle of evaporation of water drops from 72,3 ^{o} to 0 ^{o}. The spectrum of hydrogen bonds among H_2O molecules was measured in the range of -0.08– -0.1387 eV or λ = 8.9–13.8 μm using a specially designed computer program. The main estimation criterion in these studies was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in human blood serum.

2.4. Studying the Bulgarian Centinarians

Interviews have been conducted with 415 Bulgarian centenarians and long lived people and their siblings. Their heredity, body weight, health status, tobacco consumption, physical activity, attitude towards life has been analyzed. With using DNES method was performed a spectral analysis of 15 mountain water springs located in municipalities Teteven and Kuklen (Bulgaria).

The composition of water samples was studied in the laboratory of "Eurotest Control" (Bulgaria). Statistics methods were attributed to the National Statistical Institute of Bulgaria.

2.5. Studying the human blood serum

1% (v/v) solution of human blood serum was studied with the methods of IR-spectrometry, non-equilibrium (NES) and differential non-equilibrium (DNES) spectral analysis. The specimens were provided by Kalinka Naneva (Municipal Hospital, Bulgaria). Two groups of people between the ages of 50 to 70 were tested. The first group (control group) consisted of people in good clinical health. The second group included people in critical health or suffering from malignant diseases.

2.6. IR-spectroscopy

IR-spectra were registered on Brucker Vertex ("Brucker", Germany) IR spectrometer (a spectral range: average IR – 370–7800 cm^{-1}; visible – 2500–8000 cm^{-1}; the permission – 0,5 cm^{-1}; accuracy of wave number – 0,1 cm^{-1} on 2000 cm^{-1}) and on Thermo Nicolet Avatar 360 Fourier-transform IR (M. Chakarova).

2.7. Statistical Processing of Experimental Data

Statistical processing of experimental data was performed using the statistical package STATISTISA 6 using the Student's *t*- criterion (at $p < 0.05$).

3. Results and Discussions

3.1. Comparative Analysis between Longevity of Long Lived Centenarians and Their Siblings

In frames of the research 121 long living people from Bulgaria over 90 years of age have been studied together with their 294 siblings. The average lifespan of long lived people and centenarians in mountain areas is 94.1 years. For the average lifespan of long lived people in plain areas the result is 90.6 years. The most adult person from mountain areas is 104 years old and for plain areas is 97 years old. For the brothers and sisters of long live people from mountain areas the average lifespan is 88.5 years. For the brothers and sisters of long live people from plain areas the average lifespan is 86.4 years. The difference in life expectancy of the two groups of people is reliable and is at $p < 0.05$, *t*-Student's criteria at a confidence level of $t = 2.36$. There are distances of no more than 50–70 km between these places and the only difference is mountain water and air.

There have been 21519 residents in Teteven and 142 of them were born before 1924. Figure 1 shows the interrelation between the year of birth of long lived people (age) and their number (Teteven municipality, Bulgaria).

Figure 1. Interrelation between the year of birth of long lived people (age) and their number in Teteven municipality, Bulgaria

It was shown in Figure 1 that the rate of aging increases with time. In 1963 L. Orgel showed that the aging process is associated with the synthesis of abnormal proteins [7]. Figure 2 shows L. Orgel's results on the interrelation between age and number of cancer patients. The accumulation of errors in synthesis of abnormal proteins increases exponentially over time with age. Cells taken from elderly people show the reduced levels of transcription or transmission of information from DNA to RNA. Therefore, the probability of cancer increases with age. The interrelation between the number of Bulgarian centenarians in the mountainous municipality of Teteven and their age is close to exponential.

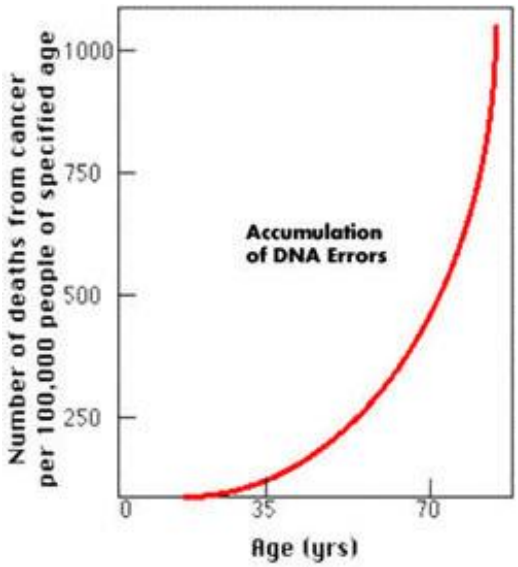

Figure 2. Interrelation between age and the number of cancer patients [7]

Here are submitted the data on longevity for Bulgaria:

1) Varna district – 44 centenarians per 1 million of inhabitants, plain and sea regions;

2) Pleven district – 78 centenarians per 1 million of inhabitants, plain regions;

3) Teteven district – 279 centenarians per 1 million of inhabitants, hills and mountainous regions;

4) Bulgaria – 47 centenarians per 1 million of inhabitants.

Analogous situation is observed in the Russian North. According to G. Berdishev, people inhabiting the Russian North – the Yakuts and the Altaians as well as the Buryats, drink mountain water obtained after the melting of ice. Altai and Buryat, Caucasus water sources in Russia are known as moderately warm, with temperatures of 8–10 ^{o}C, the water is generally ice-free in winter. This phenomenon is explained by the fact that the melt water contains a low percentage of deuterium compared with ordinary tap water that is believed to have a positive effect on the tissue cells and metabolism. Melt water in Russia is considered to be a good folk remedy for increasing physical activity of the human body, enhancing the vitality of the organism and has a beneficial effect on metabolism [8]. In the world are popular the sources with melt water from Canada, Norway, Island and Alaska.

3.2. Clinical Evidence with Human Blood Serum Testing

It was established experimentally that in the process of evaporation of water drops, the wetting angle θ decreases discreetly to zero, and the diameter of water drop basis is only slightly altered, that is a new physical effect [9]. Based on this effect, by means of measurement of the wetting angle within equal intervals of time is determined the function of distribution of H_2O molecules according to the value of f(θ). The distribution function is denoted as the energy spectrum of the water state. The theoretical research established the dependence between the surface tension of water and the energy of hydrogen bonds among individual H_2O-molecules. The hydrogen bonding results from interaction between electron-deficient H-atom of one H_2O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen

acceptor) on the neighboring H_2O molecule; the structure of hydrogen bonding may be defined as $O\cdots H^{\delta+}-O^{\delta-}$.

For calculation of the function f(E) represented the energy spectrum of water, the experimental dependence between the wetting angle (θ) and the energy of hydrogen bonds (E) is established:

$$f(E) = \frac{14{,}33 f(\theta)}{[1-(1+bE)^2]^2} \qquad (1)$$

where b = 14.33 eV^{-1}

The relation between the wetting angle (θ) and the energy (E) of the hydrogen bonds between H_2O molecules is calculated by the formula:

$$\theta = arcos\ (-1 - 14.33E) \qquad (2)$$

The energy spectrum of water is characterized by a non-equilibrium process of water droplets evaporation, therefore, the term non-equilibrium spectrum (NES) of water is used.

The difference *Δf(E) = f (samples of water) – f (control sample of water)* – is called the "differential non-equilibrium energy spectrum of water" (DNES).

Thus, the DNES spectrum is an indicator of structural changes in water, because the energy of hydrogen bonds in water samples differ due to the different number of hydrogen bonds in water samples, which may result from the fact that different waters have different structures and composition and various intermolecular interactions – various associative elements etc. [10]. The redistribution of H_2O molecules in water samples according to the energy is a statistical process of dynamics.

Figure 3 shows the average NES-spectrum of deionised water. On the X-axis are shown three scales. The energies of hydrogen bonds among H_2O molecules are calculated in eV. On the Y-axis is depicted the function of distribution of H_2O molecules according to energies f(E), measured in unit eV^{-1}. For DNES spectrum the function is Δf(E) in unit eV^{-1}. Arrow A designates the energy of hydrogen bonds among H_2O molecules, which is accepted as most reliable in spectroscopy. Arrow B designates the energy of hydrogen bonds among H_2O molecules the value of which is calculated:

$$\bar{E} = -0.1067 \pm 0.0011\ eV \qquad (3)$$

Arrow C designates the energy at which the thermal radiation of the human body, considered like an absolute black body (ABB) with a temperature 36.6 ^oC, is at its maximum. A horizontal arrow designates the window of transparency of the earth atmosphere for the electromagnetic radiation in the middle infrared range of the Sun toward the Earth and from the Earth toward the surrounding cosmic space. It is seen that the atmosphere window of transparency almost covers the energy spectrum of water.

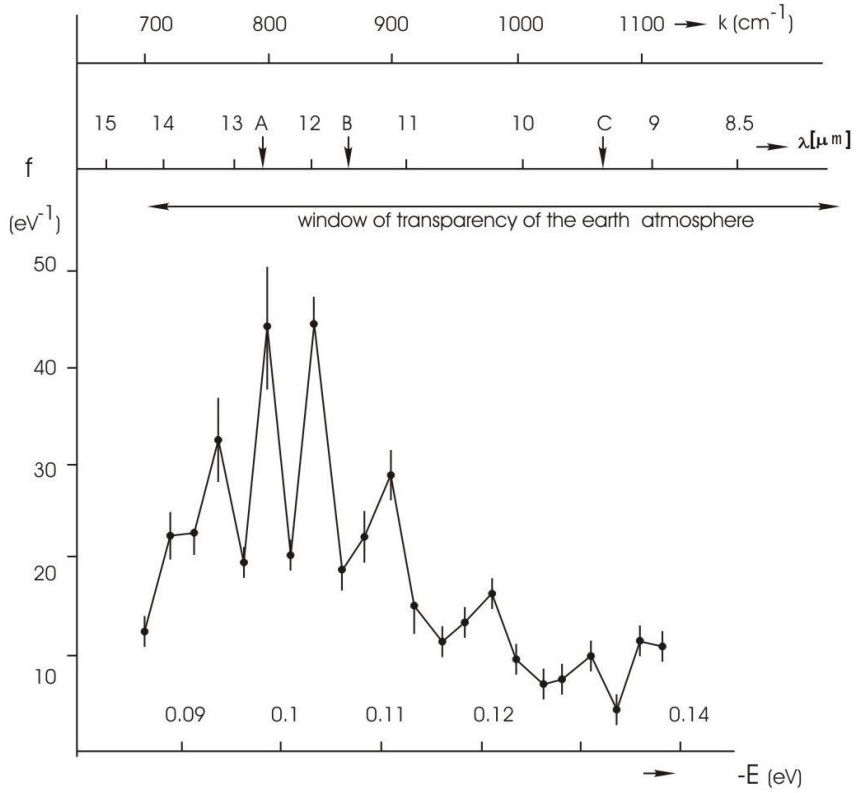

Figure 3. Non-equilibrium (NES) spectrum of deionized water (chemical purity – 99.99 %; pH – 6,5–7,5; total mineralization – 200 mg/l; electric conductivity – 10 μS/cm): the horizontal axis shows the energy of the H...O hydrogen bonds in the associates – E (eV); the vertical axis – the energy distribution function – f (eV^{-1}); *k* – the vibration frequency of the H–O–H atoms (cm^{-1}); λ – wavelength (μm)

The study of the IR spectrum of water in the composition of physiologic fluids (urine, blood, serum) can also provide data on metabolic processes in the human body and longevity, because the IR-spectrum reflects the metabolic processes. Authors have conducted studies of a 1% (v/v) solution of blood serum by spectral analysis of non-equilibrium energy (NES) spectrum and differential equilibrium energy (DNES) spectrum on two groups of people between 50 and 70 years of age. The first group consisted of people in excellent health. The second group consisted of people in a critical state and patients with malignant tumors. As a main biophysical parameter was investigated the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules in the blood serum. The result was obtained as a difference between the NES-spectrum of 1% solution of blood serum and NES-spectrum of deionized water control sample – DNES-spectrum, measured as the difference Δf(E) = f (samples of water) – f (control sample of water). The DNES-spectrum obtained from the first group has a local maximum energy ($\Delta E_{H...O}$) at -9.1±1.1 meV and from the second group -1.6±1.1 meV. The results between the two groups have a statistical difference in Student's criterion at $p < 0.05$. For the control group of healthy people the value of the largest local maximum in the DNES-spectrum was detected at E = -0.1387 eV, or at a wavelength of λ = 8.95 μm. For the group of people in a critical state and the patients with malignant tumors, the analogous values of the largest local maximums of the DNES-spectrum shifted to lower energies compared with the control group of people. Water in the human body possesses IR-spectrum that reflects the metabolic processes in the organism. It can be demonstrated by analysis of human blood serum by IR-spectroscopy. The magnitude of the largest local maximum in IR-spectrum of blood serum from healthy people of control group observed at -0.1387 eV at a wavelength – 8.95 μm. For a group of people in critical health condition and patients with malignant tumors the greatest values of local extremum in the IR-spectrum are shifted to lower energies relative to the control group. In IR-spectrum of human blood serum are detected local maxima at λ = 8.55, 8.58, 8.70, 8.77, 8.85, 9.10, 9.35 and 9.76 μm [11]. The resulting peak at λ = 8.95 μm in IR-spectrum

detected by us [12] approaching the peak at λ = 8.85 μm monitored by Russian researchers. In the control group of healthy people the average value of the energy distribution function f(E) at λ = 8.95 μm compiles 75.3 eV, and in a group of people in critical condition – 24.1 eV. The level of reliability of the results is $p< 0.05$ according to the Student's t-test. In 1995 A. Antonov performed DNES-experiments with impact on tumor mice cells in water [13]. There was a decrease of the spectrum compared with the control sample of cells from a healthy mouse. The decrease was also observed in the spectrum of human blood serum of terminally ill people relative to that of healthy people. With increasing of age of long-living blood relatives, the function of distribution of H_2O molecules according to energies at -0.1387 eV decreases. In this group of tested people the result was obtained by DNES at -5.5±1.1 meV, the difference in age was of 20–25 years in relation to the control group. It should be noted that most of Bulgarian centenarians inhabit the Rhodopes Mountains areas. Among to the DNES-spectrum of mountain waters similar to the DNES-spectrum of blood serum of healthy people at λ = 8.95 μm, was the DNES-spectrum of water in the Rhodopes. The mountain waters from Teteven, Boyana and other Bulgarian provinces have similar parameters. Tables 1, 2 and 3 show the composition of mountain springs in Teteven and Kuklen (Bulgaria) and local extremums in NES-spectra of water. The local extremums were detected at E = -0.11 eV and E = -0.1387 eV. The value at E = -0.11 eV is characteristic for the presence of Ca^{2+}. The value at E = -0.1380 eV is characteristic for inhibiting the growth of cancer cells. Experiments conducted by A. Antonov with cancer cells of mice demonstrated a reduction of this local extremum to a negative value. Analysis by the DNES-method of aqueous solutions of natural mineral sorbents – shungite (carbonaceous mineral from Zazhoginskoe deposit in Karelia, Russia) and zeolite (microporous crystalline aluminosilicate mineral from Most village, Bulgaria) showed the presence of a local maximum at E = -0.1387 eV for shungite and E = -0.11 eV for zeolite [14]. It should be noted that owing to the unique porous structures both the natural minerals shungite and zeolite are ideal natural water adsorbents effectively removing from water organochlorine compounds, phenols, dioxins, heavy metals, radionuclides, and color, and gives the water a good organoleptic qualities, additionally saturating it with micro-and macro-elements [15]. It is worth to note that in Bulgaria the main mineral deposits of Bulgarian zeolites are located in the Rhodope Mountains, whereat has lived the greatest number of Bulgarian centenarians. It is thought that water in these areas is cleared in a natural way by zeolite. Therefore, a new parameter is researched – a local extremum of energy at (-0.1362– -0.1387 eV). This value was determined by the NES-spectrum as function of distribution of individual H_2O molecules according to energy f(E). The norm has statistically reliable result for human blood serum for the control group of people having cancer at the local extremum of f(E) ~24.1 eV^{-1}. The function of distribution according to energy f(E) for tap water in Teteven is 11.8±0.6 eV^{-1}.

3.3. Composition of water in the mountain area in Teteven municipality in Stara Planina Mountain and Kuklen municipality, Rhodopes Mountain

The statistical data shows that the difference between the age of long lived people in mountain and plain areas is 3.7 years. The analyses of water sources show the differences regarding chemical composition, hardness, local extremum in NES-spectra of water eV^{-1} at (-0.1362– -0.1387 eV), isotopic shifts of D/H in water.

Tables 1, 2 and 3 show the chemical composition of mountain springs in Teteven and Kuklen (Bulgaria) and local extremums in NES-spectra of water.

Table 1. The composition of mountain water springs in Zlatishko-Tetevenska Mountain (Teteven municipality, Bulgaria) and local extremums in NES-spectra of water

Indicators	Results of the research (mg/l)	Norm
Sodium (Na^+)	0.96	<200
Calcium (Ca^{2+})	100.4	<150
Magnesium (Mg^{2+})	12.65	<80
Iron (Fe)	0.016	<0.2
Manganese (Mn^{2+})	0.0018	<0.2
Zinc (Zn^{2+})	0.18	<4.0
Sulfates (SO_4^{2-})	81.8	<250
Chlorides (Cl^-)	3.96	<250
Carbonates (CO_3^{2-})	<2.0	–
Hydrocarbonates (HCO_3^-)	184.0	–
Other values	Results	
Active reaction (pH)	7.9 alkaline	6.5–9.5
Electroconductivity	536.8 μS/cm	<2000
Hardness of water	16.5 dH hard	<33.7
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	36.9	>24.1

*Function of distribution of H_2O molecules according to energy f(E)

Table 2. The composition of mountain water springs in Vasiliovska Mountain (Teteven municipality, Bulgaria) and local extremum in NES-spectra of water.

Indicators	Results of the research (mg/l)	Norm
Sodium (Na^+)	4.5	<200
Calcium (Ca^{2+})	55.5	<150
Magnesium (Mg^{2+})	2.28	<80
Iron (Fe)	0.0127	<0.2
Manganese (Mn^{2+})	0.0014	<0.2
Zinc (Zn^{2+})	0.006	<4.0
Sulfates (SO_4^{2-})	16.9	<250
Chlorides (Cl^-)	3.4	< 250
Carbonates (CO_3^{2-})	< 2.0	–
Hydrocarbonates (HCO_3^-)	118.0	–
Other values	Results	
Active reaction (pH)	7.4 alkaline	6.5–9.5
Electroconductivity	285.0 μS/cm	<2000
Hardness of water	7.9 dH slightly hard	<33.7
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	40.1	>24.1

*Function of distribution of H_2O molecules according to energy f(E)

Table 3. The composition of mountain water spring Eco Hotel Zdravetz, Rhodopes Mountain (Kuklen municipality, Bulgaria) and local extremum in NES-spectra of water

Indicators	Results of the research (mg/l)	Norm
Sodium (Na^+)	7.6	<200
Calcium (Ca^{2+})	3.5	<150
Magnesium (Mg^{2+})	0.63	<80
Iron (Fe)	0.007	<0.2
Manganese (Mn^{2+})	0.002	<0.2
Zinc (Zn^{2+})	0.007	<4.0
Sulfates (SO_4^{2-})	26.8	<250
Chlorides (Cl^-)	3.00	<250
Carbonates (CO_3^{2-})	<2.0	–
Hydrocarbonates (HCO_3^-)	21.3	–
Other values	Results	
Active reaction (pH)	5.93 normal	6.5–9.5
Electroconductivity	536.8 μS/cm	<2000
Hardness of water	1.4 dH soft	<33.7
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	59.3	>24.1

*Function of distribution of H_2O molecules according to energy f(E)

The research shows the results of water composition in field area of Dolni Dabnik. The results are: hydrocarbonates (HCO_3^-) – 184.4 mg/l, sulfates (SO_4^{2-}) – 19.2 mg/l, chlorides (Cl^-) – 9.2 mg/l, calcium (Ca^{2+}) – 50.6 mg/l, sodium (Na^+) – 14.2 mg/l. The hardness makes up 26.2 dH – it is very hard water. The maximum peak in NES-spectra of water (eV^{-1}) at (-0.1362–-0.1387 eV) in water of Danubian Plain is 23.2 eV^{-1} and in Thracian Valley is detected at -21.3 eV^{-1}. In water samples from the Danubian Plain and Thracian Valley there are data for the presence of nitrites (NO_2), nitrates (NO_3), ammonia (NH_4), phosphates (HPO_4) more than norm.

Table 4 shows optimal chemical composition of water, hardness, local extremum eV^{-1} at (-0.1362–-0.1387 eV), hardness and total mineralization of water as middle result of different studies. The areas are between 600 m and 1300 m attitude in Bulgaria and from Caucasus, Russia. In these areas are living long lived people.

Table 4. Optimal chemical composition of water, hardness, local extremum eV^{-1} at (-0.1362–-0.1387 eV) and total mineralization of water

Indicators	Results of melt and mountain water (Bulgaria) (mg/l)	Results of melt water (Russia) (mg/l)
Sodium(Na^+) + Potassium (K^+)	6.1	<30
Calcium (Ca^{2+})	29.5	<50
Magnesium (Mg^{2+})	1.5	<10
Iron (Fe)	0.083	–
Manganese (Mn^{2+})	0.0017	–
Zinc (Zn^{2+})	0.007	–
Sulfates (SO_4^{2-})	21.9	<100
Chlorides (Cl^-)	3.2	<70
Carbonates (CO_3^{2-})	<2.0	–
Hydrocarbonates (HCO_3^-)	69.7	<100

Other values	Results	
Active reaction (pH)	6.7 normal	6.5-7.0
Electroconductivity	410.9 μS/cm	<2000
Hardness of water	4.65 dH Moderately soft	<33.7
Total mineralization (g/l)	0.132	<0.3
Local extremum* eV^{-1} at (-0.1362–-0.1387 eV)	49.7	>24.1

*Function of distribution of H_2O molecules according to energy f(E)

3.4. Effects of Calcium, Magnesium, Zinc and Manganese in water on biophysical and biochemical processes in the human body

The research into distribution of local extremums (eV^{-1}) in spectra of various water samples as a function of distribution of H_2O molecules according to energy f(E) at λ = 8.95 μm shows the analogue extremum at analogous values of f(E), E and λ, which was detected in water with Ca^{2+} ions earlier demonstrated inhibiting the growth of cancer cells. Magnesium (Mg^{2+}), zinc (Zn^{2+}) and manganese (Mn^{2+}) ions dissolved in water have influence on enzymes, which are antioxidants [16]. The research of China team was categorized three groups of elements from the rice and drinking water according to their effect on longevity: Sr, Ca, Al, Mo, and Se, which were positively correlated with longevity: Fe, Mn, Zn, Cr, P, Mg, and K, which had a weak effect on local longevity, and Cu and Ba, which had a negative effect on longevity [17]. There was a positive correlation between the eSOD activity and the age and a negative correlation between the eSOD activity and concentration of Zn^{2+} in plasma. An inverse correlation was also found between the content of Zn^{2+} ions in plasma relative to the age. The prevalence of Zn^{2+} deficiency is increased with age; with normal Zn^{2+} levels it is observed in about 80% of adult people and only in 37 % of the non-agenarians. Aging is an inevitable biological process that is associated with gradual and spontaneous biochemical and physiological changes and the increased susceptibility to diseases. Because the nutritional factors are involved in improving the immune functions, metabolic balance, and antioxidant defense, some nutritional factors, such as Zn, may modify susceptibility to disease and promote healthy aging. In *vitro* (human lymphocytes exposed to endotoxins) and *in vivo* (old or young mice fed with low zinc dietary intake) studies revealed that zinc is important for immune efficiency (innate and adaptive), antioxidant activity (superoxide dismutase), and cell differentiation *via* clusterin/apolipoprotein J. The intracellular Zn homeostasis is regulated by metallothioneins (MT) *via* an ion release through the reduction of thiol groups in the MT molecule [18]. Zinc in composition of water improves the antioxidative enzymes in red blood cells [19].

The magnesium deficiency and oxidative stress have both been identified as pathogenic factors in aging and in several age-related diseases. The link between these two factors is unclear in humans although, in experimental animals, severe Mg^{2+} deficiency has been shown to lead to the increased oxidative stress [20]. The antioxidants against free radical damage include tocopherol (vitamin E), ascorbic acid (vitamin C), β-carotene, glutathione, uric acid, bilirubin, and several metalloenzymes including glutathione peroxidase (Se), catalase (Fe), and superoxide dismutase (Cu, Zn, Mn) and proteins such as ceruloplasmin (Co). The extent of the tissue damage is the result of the balance between the free radicals generated and the antioxidant protective defense system [21]. The norm in water for Zn^{2+} and Mg^{2+} according to the World Health Organization (WHO) should be less than 20 μg. For the Na^{+} content the norm according to the WHO is less than 20 mg.

The interesting results on the concentration of Ca^{2+} in water were obtained in USA and Canada. According to the statistical information the most number of centenarians in Canada per 1 million of population is observed in Nova Scotia (210 of centenarians per 1 million). In water from Nova Scotia the Ca^{2+} content makes up 6.8 mg/l. N. Druzhyak, Russia showed that in the places wherein live the most number of centenarians the Ca^{2+} content in water was 8–20 mg/l. The only risk factor regarding the increased Ca^{2+} content in water is cardiovascular diseases [22].

The following reactions occur in water if there are high concentrations of Ca^{2+} and Mg^{2+} ions: the reaction of limestone ($CaCO_3$) and gypsum ($CaSO_4 \cdot 2H_2O$) with water to separate the calcium (Ca^{2+}), carbonates (CO_3^{2-}) and sulfate (SO_4^{2-}) ions. By increasing the mineralization of water the content of Ca^{2+} ions decreases. During the concentration of the solutions Ca^{2+} ions are precipitated. With the increase of carbon dioxide (CO_2) in water and decreasing of the pH value the content of

Ca^{2+} increases. The reaction of interaction of dolomite ($CaCO_3 \cdot MgCO_3$) with water makes the formation of Mg^{2+} ions. Hydrocarbonates (HCO_3^-) and carbonates (CO_3^{2-}) ions are formed by reaction of interaction of karst rocks, CO_2 and water.

3.5. The measurement of deuterium content in mountain and melt water

Preliminary analyses of water from various water sources show that mountain and melt water as the result of natural isotope purification contains less amount of deuterium. This water also contains ions of Ca^{2+}, Mg^{2+}, Na^+, HCO_3^- and SO_4^{2+}. The content of K^+ and N^+ cations in the melt water are <30 mg/l, Mg^{2+} – <10 mg/l, Ca^{2+} – <50 mg/l, the content of SO_4^{2-} – <100 g/l, HCO_3^- <100 mg/l, Cl^- – less than 70 mg/l, total rigidity ≤ 5 mEq/l, the total mineralization ≤ 0.3 g/l, pH – 6.5–7.0 at 25 °C (Table 5). The degree of natural purification of melt water from impurities makes up ~50–60%. The concentration of salts of rigidity – Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, heavy metals and organochlorine compounds, as well as heavy isotopes, including deuterium in melt water is less that of ordinary portable water. This fact is important because some authors consider the hardness of the water to be among the main factors in cardiovascular diseases. However, mild correlation was further proven that water hardness could not be a decisive factor for human longevity.

Table 5. Chemical composition of melt water obtained from tap water by the freeze-thaw method

Cations, mg/l	
$K^+ + Na^+$	20–25
Mg^{2+}	5–10
Ca^{2+}	25–30
Anions, mg/l	
SO_4^{2-}	<90
HCO_3^-	50–100
Cl^-	<50
Other physical characteristics	
Total rigidity, mEq/l	≤5
Total mineralization, g/l	≤0,2
pH at t =+25 °C	6.5–7.0
Deuterium content, ppm	~129.5

The analyses of water from various sources of Russia and Bulgaria show that the mountain water contains on average ~2–4% less deuterium in form of HDO, than the river water and sea water. In natural waters, the deuterium content is distributed irregularly: from 0.02–0.03 mol.% for river and sea water, to 0.015 mol.% for water of Antarctic ice – the most purified from deuterium natural water containing deuterium in 1.5 times less than that of seawater. According to the international SMOW standard isotopic shifts for D and ^{18}O in sea water: $D/H = (155.76 \pm 0.05) \cdot 10^{-6}$ (155.76 ppm) and $^{18}O/^{16}O = (2005.20 \pm 0.45) \cdot 10^{-6}$ (2005 ppm) [23]. For SLAP standard isotopic shifts for D and ^{18}O in seawater: $D/H = 89 \cdot 10^{-6}$ (89 ppm) and for a pair of $^{18}O/^{16}O = 1894 \cdot 10^{-6}$ (1894 ppm). In surface waters, the ratio $D/H = \sim(1.32–1.51) \cdot 10^{-4}$, while in the coastal seawater – $\sim(1.55–1.56) \cdot 10^{-4}$. Waters of other underground and surface water sources contain varied amounts of deuterium (isotopic shifts) – from δ = +5,0 D,%, SMOW (Mediterranean Sea) to to δ = -105 D,%, SMOW (Volga River). The natural waters of CIS countries are characterized by negative deviations from SMOW standard to $(1.0–1.5) \cdot 10^{-5}$, in some places up to $(6.0–6.7) \cdot 10^{-5}$, but there are observed positive deviations at $2.0 \cdot 10^{-5}$. The content of the lightest isotopomer – $H_2{}^{16}O$ in water corresponding to SMOW standard is 997.0325 g/kg (99.73 mol.%), and for SLAP standard – 997.3179 g/kg (99.76 mol.%).

The thawed snow and glacial water in the mountains and some other regions of the Earth also contain less deuterium than ordinary drinking water. On average, 1 ton of river water contains 150–200 g deuterium [24]. The average ratio of H/D in nature makes up approximately 1:5700. According to the calculations, the human body throughout life receives about 80 tons of water

containing in its composition 10–12 kg of deuterium and associated amount of heavy isotope ^{18}O. That is why it is so important to purify water from heavy isotopes of D and ^{18}O.

The local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state; the substitution with deuterium changes the vibrational-rotational transitions in H_2O molecule that is why it appears other local maximums in IR-spectra. In the water vapor state, the vibrations involve combinations of symmetric stretch (υ_1), asymmetric stretch (υ_3) and bending (υ_2) of the covalent bonds with absorption intensity (H_2O) $\upsilon_1;\upsilon_2;\upsilon_3$ = 2671; 1178.4; 2787.7 cm^{-1}. For liquid water absorption bands are observed in other regions of the IR-spectrum, the most intense of which are located at 2100, cm^{-1} and 710–645 cm^{-1}. For D_2O molecule these ratio compiles 2723.7, 1403.5 and 3707.5 cm^{-1}, while for HDO molecule – 2671.6, 1178.4 and 2787.7 cm^{-1}. HDO (50 mole% H_2O + 50 mole% ^2H_2O; ~50 % HDO, ~25 % H_2O, ~25 % D_2O) has local maxima in IR-spectra at 3415 cm^{-1}, 2495 cm^{-1} 1850 cm^{-1} and 1450 cm^{-1} assigned to OH^- -stretch, OD^- -stretch, as well as combination of bending and libration and HDO bending respectively. The local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state because at changing the atomic mass of hydrogen and deuterium atoms in the water molecule their interaction will also change, although the electronic structure of the molecule and its ability to form H-bonds, however, remains the same; with the substitution with deuterium the vibrational-rotational transitions are changed, that is why it appears other local maximums in IR-spectra. The result is reliable regarding the content of deuterium in natural waters from 0.015–0.03%.

In the IR-spectrum of liquid water absorbance band considerably broadened and shifted relative to the corresponding bands in the spectrum of water vapor. Their position depends on the temperature [25]. However, the temperature dependence of individual spectral bands of liquid water is very complex [26]. Furthermore, the complexity of the IR-spectrum in the area of OH^- stretching vibration can be explained by the existence of different types of H_2O associations, manifestation of overtones and composite frequencies of OH^- groups in the hydrogen bonds, and the tunneling effect of the proton (for relay mechanism) [27]. Such complexity makes it difficult to interpret the spectrum and partly explains the discrepancy in the literature available on this subject.

In liquid water and ice the IR-spectra are far more complex than those ones of the vapor due to vibrational overtones and combinations with librations (restricted rotations, e.g. rocking motions). These librations are due to the restrictions imposed by hydrogen bonding (minor L_1 band at 395.5 cm^{-1}; major L_2 band at 686.3 cm^{-1}; for liquid water at 0 ^oC, the absorbance of L_1 increasing with increasing temperature, while L_2 absorbance decreases but broadens with reduced wave number with increasing temperature [28]. The IR spectra of liquid water usually contain three absorbance bands, which can be identified on absorption band of the stretching vibration of OH^- group; absorption band of the first overtone of the bending vibration of the molecule HDO and absorption band of stretching vibration of OD^- group. Hydroxyl group OH^- is able to absorb much infrared radiation in the infrared region of the IR-spectrum. Because of its polarity, these groups typically react with each other or with other polar groups to form intra-and intermolecular hydrogen bonds. The hydroxyl groups, which are not involved in formation of hydrogen bonds, usually produce the narrow bands in IR spectrum, while the associated groups – broad intense absorbance bands at lower frequencies. The magnitude of the frequency shift is determined by the strength of the hydrogen bond. Complication of the IR spectrum in the area of OH^- stretching vibrations can be explained by the existence of different types of associations of H_2O molecules, a manifestation of overtones and combination frequencies of OH^- groups in hydrogen bonding, as well as the proton tunneling effect (on the relay mechanism).

An assignment of main absorption bands in the IR-spectrum of liquid water is given in (Table 6). The IR spectrum of H_2O molecule was examined in detail from the microwave till the middle (4–17500 cm^{-1}) visible region and the ultraviolet region – from 200 nm^{-1} to ionization limit at 98 nm^{-1} [29]. In the middle visible region at 4–7500 cm^{-1} are located rotational spectrum and the bands corresponding to the vibrational-rotational transitions in the ground electronic state. In the ultraviolet region (200 nm^{-1} to 98 nm^{-1}) are located bands corresponding to transitions from the excited electronic states close to the ionization limit in the electronic ground state. The intermediate region of the IR-spectrum – from 570 nm to 200 nm corresponds to transitions to higher vibrational levels of the ground electronic state.

The results of IR-spectroscopy with device Infra Spec VFA-IR show that at 4.1 μm, even at low concentrations of deuterium of 0.35 and 0.71%, there is observed a decline in the local maximums relative to the local maximum of 100% pure water (the local maximums in IR-spectra reflect vibrational-rotational transitions in the ground electronic state because at changing the atomic mass of hydrogen and deuterium atoms in the water molecule their interaction will also change, although the electronic structure of the molecule and its ability to form H-bonds, however, remains the same; with the substitution with deuterium the vibrational-rotational transitions are changed, that is why it appears other local maximums in IR-spectra. The result is reliable regarding the content of deuterium in natural waters from 0.015–0.03%.

Table 6. The assignment of main frequencies in IR-spectra of H_2O and D_2O

Main vibrations of liquid H_2O and ^2H_2O				
Vibration(s)	H_2O (t = +25 °C)		D_2O (t = +25 °C)	
	υ, cm^{-1}	E_0, M^{-1} cm^{-1}	υ, cm^{-1}	E_0, M^{-1} cm^{-1}
Spinning ν_1 + deformation ν_2	780-1645	21.65	1210	17.10
Composite $\nu_1 + \nu_2$	2150	3.46	1555	1.88
Valence symmetrical ν_1, valence asymmetrical ν_3, and overtone $2\nu_2$	3290-3450	100.65	2510	69.70

At further transition from H_2O monomers to H_4O_2 dimmer and H_6O_3 trimmer absorption maximum of valent stretching vibrations of the O-H bond is shifted toward lower frequencies (υ_3 = 3490 cm^{-1} and υ_1 = 3280 cm^{-1}) [30] and the bending frequency increased (υ_2 = 1644 cm^{-1}) because of hydrogen bonding. The increased strength of hydrogen bonding typically shifts the stretch vibration to lower frequencies (red-shift) with greatly increased intensity in the infrared due to the increased dipoles. In contrast, for the deformation vibrations of the H–O–H, it is observed a shift towards higher frequencies. Absorption bands at 3546 and 3691 cm^{-1} were attributed to the stretching modes of the dimmer [$(H_2O)_2$]. These frequencies are significantly lower than the valence modes of ν_1 and ν_3 vibrations of isolated H_2O molecules at 3657 and 3756 cm^{-1} respectively). The absorption band at 3250 cm^{-1} represents overtones of deformation vibrations. Among frequencies between 3250 and 3420 cm^{-1} is possible Fermi resonance (this resonance is a single substitution of intensity of one fluctuation by another fluctuation when they accidentally overlap each other). The absorption band at 1620 cm^{-1} is attributed to the deformation mode of the dimmer. This frequency is slightly higher than the deformation mode of the isolated H_2O molecule (1596 cm^{-1}). A shift of the band of deformation vibration of water in the direction of high frequencies at the transition from a liquid to a solid state is attributed by the appearance of additional force, preventing O–H bond bending. Deformation absorption band in IR-spectrum of water has a frequency at 1645 cm^{-1} and very weak temperature dependence. It changes little in the transition to the individual H_2O molecule at a frequency of 1595 cm^{-1}. This frequency is found to be sufficiently stable, while all other frequencies are greatly affected by temperature changes, the dissolution of the salts and phase transitions. It is believed that the persistence of deformation oscillations is stipulated by processes of intermolecular interactions, e.g. by the change in bond angle as a result of interaction of H_2O molecules with each other, as well as with cations and anions.

Thus the study of the characteristics of the IR spectrum of water allows to answer the question not only on the physical parameters of the molecule and the covalent bonds at isotopic substitution with deuterium, but also to make a certain conclusion on associative environment in water. The latter fact is important in the study of structural and functional properties of water associates and its isotopomers at the isotopic substitution with deuterium. The substitution of H with D affects the stability and geometry of hydrogen bonds in an apparently rather complex way and may, through the changes in the hydrogen bond zero-point vibration energies, alter the conformational dynamics of hydrogen (deuterium)-bonded structures of DNA and proteins in D_2O [31] (Cleland, 1976). It may cause disturbances in the DNA-synthesis, leading to permanent changes on DNA structure and consequently on cell genotype [32, 33].

Our experiments demonstrated that the effects of deuterium on the cell possess a complex multifactor character connected to changes of physiological parameters – magnitude of the *lag*-period, time of cellular generation, outputs of biomass, a ratio of amino acids, protein, carbohydrates and fatty acids synthesized in D_2O, and with an evolutionary level of organization of investigated object as well [34, 35]. The cell evidently implements the special adaptive mechanisms promoting functional reorganization of work of the vital systems in the presence of D_2O.

4. Conclusion

In frames of this research 415 people living in the municipalities of Teteven, Yablanitza. Ugarchin, Lukovit, Lovech district; Dolni Dabnik, Pleven district, Kuklen, Pleven district (Bulgaria), where is lived the most number of long lived people and their siblings, were studied. They have the same heredity, but have lived under different conditions. The research shows that the direct relationship of man and nature – clean air, natural food from eco-farms and physical activity explains the difference between the larger number of long lived people and centenarians who live in the mountain regions of Bulgaria and Russia and their high average number. Natural mountain and melt water with chemical composition, less deuterium seems to be one of the most important factors for longevity. In Bulgaria, most long lived people and centenarians live in the Rhodope Mountains, while in Russia – in Dagestan and Yakutia. It worth to note that IR-spectrum of mountain water is most similar to the IR-spectrum of blood serum of healthy group of people with a local maximum at $\lambda = 8.95$ μm. Similar spectral characteristics possess mountain water from Teteven and other Bulgarian sources. Thus, the phenomenon of longevity is a complex phenomenon involving both genetic and phenotypic characteristics of the organism to external factors and environment – free radicals, radiation, heavy isotopes, as well as the structure and the isotopic composition of drink water. Other longevity factors are living area, health status, body mass, gender and heredity. Studying the human blood serum by NES and DNES-methods show that by measuring the average energy of hydrogen bonds among H_2O molecules and the distribution function of H_2O molecules on energies it is possible to show a vital state status of a person and associated life expectancy. These data indicate that water in the human body has the IR-spectrum resembling the IR-spectrum of human blood serum. On the characteristics of the IR-spectrum of water exerts an influence also the presence of deuterium. In the research was studied an optimal composition of mountain and melt water from areas where were lived the long live people and centenarians. There are also new proofs for biophysical and biochemical effects of calcium, magnesium, zinc and manganese in water.

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References:

1. Ignatov I. Water in the Human Body is Information Bearer about Longevity / in: *Conference on the Physics, Chemistry and Biology of Water*. – NY: Vermont Photonics, 2012.

2. Ignatov I., Mosin O.V. Possible Processes for Origin of Life and Living Matter with Modeling of Physiological Processes of Bacterium *Bacillus subtilis* in Heavy Water as Model System // *Journal of Natural Sciences Research*. 2013. Vol. 3(9). P. 65–76.

3. Ignatov I., Mosin O.V. Modeling of Possible Processes for Origin of Life and Living Matter in Hot Mineral and Seawater with Deuterium // *Journal of Environment and Earth Science*. 2013. Vol. 3(14). P. 103–118.

4. Mosin O., Ignatov I., Skladnev D., Shvets V. Studying of Phenomenon of Biological Adaptation to Heavy Water // *European Journal of Molecular Biotechnology*. 2014. Vol. 6. № 4. P. 180–209.

5. Ignatov I., Mosin, O.V. Structural Mathematical Models Describing Water Clusters // *Journal of Mathematical Theory and Modeling*. 2013. Vol. 3(11). P. 72–87.

6. Ignatov I., Mosin O.V. Isotopic Composition of Water as Main Factor for Longevity // *Drug Development and Registration*. 2014. Vol. 9(4). P. 146–155 [in Russian].

7. Orgel L. The maintenance of the accuracy of protein synthesis and its relevance to aging // *Biochemistry*. 1963. Vol. 49. P. 517–521.

8. Mosin O., Ignatov I. Biological Adaptation of Organisms in Heavy Water // *Journal of Health, Medicine and Nursing*. 2014. Vol. 7. P. 101–140**.**

9. Antonov A. *Research of the Non-equilibrium Processes in the Area in Allocated Systems*. Dissertation thesis for degree "Doctor of physical sciences". – Blagoevgrad, Sofia, 1995.

10. Ignatov I., Mosin O.V. Methods for Measurements of Water Spectrum. Differential Non-equilibrium Energy Spectrum Method (DNES) // *Journal of Health, Medicine and Nursing***.** 2014. Vol. 6. P. 50–72.

11. Krasnov V.V., Gordetsov A.S. Infrared spectral analysis of blood serum as level of disturbances of metabolic processes in infusion children pathology // *Clinical Medicine*. 2009. P. 83–94 [in Russian].

12. Ignatov I., Mosin O.V., Velikov B. Mountain Water as a Factor of Human Longevity. Local Extremum at 8.95 μm in Spectrum of Water as Indicator for Health and Longevity // *Journal of Medicine, Physiology and Biophysics*. 2015. Vol. 9. P. 51–81.

13. Antonov A., Yuskesselieva L. Selective high frequency discharge (Kirlian effect) // *Acta Hydrophysica*. 1985. Vol. 5. P. 29–31.

14. Ignatov I., Mosin O.V. The Structure and Composition of Carbonaceous Fullerene Containing Mineral Shungite and Microporous Crystalline Aluminosilicate Mineral Zeolite. Mathematical Model of Interaction of Shungite and Zeolite with Water Molecules // *Advances in Physics Theories and Applications*. 2014. Vol. 28. P. 10–21.

15. Mosin O.V., Ignatov I. The structure and composition of natural carbonaceous fullerene containing mineral shungite // *International Journal of Advanced Scientific and Technical Research*. 2013. Vol. **6**(11–12). P. 9–21.

16. Ignatov I., Mosin O.V., Velikov B. Longevity Factors and Mountain Water of Bulgaria in Factorial Research of Longevity // *Journal of Medicine, Physiology, Biophysics*. 2014. Vol. 1. P. 13–33.

17. Lv. J., Wang W., Krafft T., Li Y., Zhang F., Yuan F. Effects of Several Environmental Factors on Longevity and Health of the Human Population of Zhongxiang, Hubei // *China Biol. Trace Elem. Res*. 2011. Vol. 143(2). P. 702–716.

18. Mocchegiani E. Zinc, Metallothioneins, and Longevity - Effect of Zinc Supplementation: Zincage Study // *Ann N. Y. Acad. Sci*. 2007. Vol. 1119. P. 129–146.

19. Malhotra A., Dhawan D.K. Zinc Improves Antioxidative Enzymes in Red Blood Cells and Hematology in Lithium-Treated rats // *Nutr. Res*. 2008. Vol. 28(1). P. 43–50.

20. Begona M. Magnesium Status and Parameters of the Oxidant-Antioxidant Balance in Patients with Chronic Fatigue: Effects of Supplementation with Magnesium // *Journal of American College for Nutrition*. 2000. Vol. 19(3). P. 374–382.

21. Mariani E. Antioxidant Enzyme Activities in Healthy Old Subjects: Influence of Age, Gender and Zinc Status: Results from the Zincage Project // *Biogerentology*. 2006. Vol. 7(5–6). P. 391–398.

22. Simon M. How Much Calcium Is in Your Drinking Water? A Survey of Calcium Concentrations in Bottled and Tap Water and Their Significance for Medical Treatment and Drug Administration // *HSS Journal*. 2006. Vol. 2(2). P. 130–135.

23. Lis G., Wassenaar L.I., Hendry M.J. High-precision Laser Spectroscopy D/H and $^{18}O/^{16}O$ Measurements of Microliter Natural Water Samples // *Anal. Chem*. 2008. Vol. 80(1). P. 287–293.

24. Ignatov I., Mosin O.V., Bauer E. Vortex Power Spring Water: Physical-Chemical Qualities of this Water compared to Mountain and Melt Water from Bulgaria, Russia and Glacier Rosenlaui from Swiss Alps // *Advances in Physics Theories and Applications*. 2015. Vol. 45. P. 6–29 .

25. Ignatov I., Mosin O.V. Structural Models of Water and Ice Regarding the Energy of Hydrogen Bonding // *Nanotechnology Research and Practice*. 2015. Vol. 7. № 3. P. 96–118.

26. Zelsmann H.R. Temperature dependence of the optical constants for liquid H_2O and D_2O in the far IR region // *J. Mol. Struct*. 1095. Vol. 350. P. 95–114.

27. Yukhnevitch G.B. *Infrared spectroscopy of water*. – Moscow: Nauka, 1973. 207 p. [in Russian].

28. Brubach J.B., Mermet A., Filabozzi A., Gerschel A., Roy P. Signatures of the Hydrogen Bonding in the Infrared Bands of Water // *J. Chem. Phys.,* 2005. Vol. 122. P. 184509.

29. Walrafen G.E. Raman and infrared spectral investigations of water structure / In: *Water a Comprehensive Treatise*, F. Franks, Ed. – New York: Plenum Press, 1972, Vol. 1. pp. 151–214.

30. Eisenberg D., Kauzmann W. *The Structure and Properties of Water*. – London: Oxford University Press, 1969.

31. Cleland W.N. *Isotope effects on enzyme-catalyzed reactions*. W.N. Cleland, M.N. O'Leary & D.D. Northrop (eds.) – Baltimore, London, Tokyo, University Park Press, 1976. 303 p.

32. Török G., Csík M., Pintér A. Effects of Different Deuterium Concentrations of the Media on the Bacterial Growth and Mutagenesis. *Egészségtudomány // Health Science*. 2000. Vol. 44. P. 331–338.

33. Lamprecht I., Schroeter D., Paweletz N. Disorganization of Mitosis in HeLa Cells by Deuterium oxide // European journal of cell biology. 1989. Vol. 50(2). P. 360–369.

34. Mosin O., Ignatov I. The Biological Adaptation to Deurerium Oxide. Phenotypic or Genotypic Phenomenon? // *Journal of Medicine, Physiology and Biophysics*. 2015. Vol. 16. P. 12–28.

35. Mosin O.V., Ignatov I. Studying of isotopic effects of heavy water in biological systems on example of prokaryotic and eukaryotic cells // *Biomedicine, Moscow*. 2012. Vol. 1(1–3). P. 31–50 [in Russian].

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Изучение состава и свойств горной и талой воды Болгарии и России как факторов долголетия. Эффекты катионов кальция, магния, цинка и марганца в воде на организм человека

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Аннотация

Показано, что горная и талая вода являются важными факторами долголетия. К другим факторам относятся наследственность, пол, масса тела, питание, психологический статус, семейные отношения. Природные воды, полученные из различных болгарских родников, а также талая вода и сыворотка крови больных раком людей 50-70 лет были исследованы с помощью ИК-, НЭС- и ДНЭС-методов. Мы применили НЭС- и ДНЭС-методы для расчета средней энергии водородных связей ($\Delta E_{H...O}$) между молекулами H_2O в образцах, а также распределение молекул H_2O процентов по энергиям водородных связей в интервале энергий (от -0.08 до -0.1387 эВ). Как фактор оценки измеряли значения средней энергии водородных связей ($\Delta E_{H...O}$) между молекулами H_2O, а также локальных экстремумов в ДНЭС- и ИК-спектрах различных образцов воды и сыворотки крови человека, детектируемые при E = -0,1387 эВ и λ = 8,95 мкм. Для группы людей в критическом состоянии жизни и больных со злокачественными опухолями наибольшие значения локальных экстремумов в ДНЭС-спектрах смещены в сторону меньших энергий по сравнению с контрольной группой. ИК-спектр горной воды наиболее близок к ИК-спектру сыворотки крови группы здоровых людей с локальным максимумом λ = 8.95 μm. Природная горные и талая вода с уникальным химическим составом элементов и меньшим содержанием дейтерия рассматривается нами как один из самых важных факторов долголетия. В Болгарии, наибольшее количество долгожителей живут в горах Родопы, в то время как в России – в Дагестане и Якутии. Аналогичные характеристики имеет горная вода из Тетевен и других болгарских источников. Получены новые доказательства биофизических и биохимических эффектов Ca^{2+}, Mg^{2+}, Zn^{2+} и Mn^{2+} в воде.

Ключевые слова: долголетие, горная вода, талая вода, ИК-спектроскопия, НЭС, ДНЭС.

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Research on the effects of the 'Dance of the Spiral' methodology upon the physiological parameters of plants and the essential oil content

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Abstract

"The Dance of the Spiral" is the original methodology consisting of physical exercises that based on ancient health and longevity practices. A research has been done in which this methodology has been applied to plants. Eight plants were chosen including common yarrow, wood violet, dandelion, common chicory, shepherd's purse, cranesbill, broadleaf plantain and snowdrop, each of them grows the best in one of the eight directions. The planting was performed in eight directions, since each exercise supposedly has an effect on the state of health. Two circles were empirically defined – the Spiral and the Antispiral. Further clinical trials with 20 volunteers suffered from neurological, gastro-intestinal, cardio-vascular, and articular conditions and diseases, who stayed consecutively in the Antispiral and Spiral plant circles for 6 min in each circle were performed based on their subjective assessment of their state of health and their experience after their being stayed in the Antispiral and Spiral circles as a relaxing effect (the Antispiral circle) and a stimulating effect (the Spiral circle). The research involves the composition of essential oil extracts by ^{1}H NMR, gas chromatography and gas chromatography-mass spectrometry as well as the spectral analysis by the methods of NES and DNES of water extracts from the common plant (*Achillea millefolium*) from the control group growing in its natural habitat and from the plants grown correspondingly in the Spiral and Antispiral circles. The result in the sample of common yarrow from the Spiral circle is an increase of the average energy of hydrogen bonds between H_2O molecules. The result in the sample of common yarrow from the Antispiral circle is a decrease of the average energy of hydrogen bonds between H_2O molecules. The essential oil composition of the samples planted in the Spiral and Antispiral circles is not identical in the quantitative and qualitative composition regarding the 83 components detected in them. The amount of chamazulene in the control group sample was 5.41 %; in the Spiral sample – 4.32 %; in the Antispiral sample – 10.25 %; i.e. the amount of chamazulene in the essential oil from the Antispiral sample is almost twice of that in the control group and Spiral samples.

Keywords: *Achillea millefolium*, essential oils, NES, DNES, ^{1}H NMR.

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1. Introduction

Essential oils are volatile natural organic compounds, with a characteristic smell and oil taste, insoluble in water, mostly colorless or slightly colored liquids, on which can be judged on a variety of physiological processes in plants. Essential oils are synthesized only in plants and have extremely strong physiological and pharmacological properties. Each of them represents a mixture of several individual isoprene chemical compounds – terpenes – carbohydrates with isoprene (C_5H_8) as the major building block and their derivatives (terpenoids). Essential oils are volatile, dissolve in lipoid solvents, and have characteristic scents. The composition of essential oils depends on the type of plant, its chemotype, weather conditions in the year of collection, storage conditions of raw materials, the extraction method of essential oils, as well as the duration and storage conditions [1].

Essential oils are secreted in plants by special structures on a plant called 'receptacles'. According to their location, receptacles are divided into two groups:

- External (exogenous) – simple glandular hairs, complex glandular hairs, glandular scales, and glandular spots;
- Internal (endogenous) – excretory cells, schizogennite receptacles, mixed schizo-lizogennite receptacles.

Essential oils are the active metabolites of metabolic processes occurring in the plant cells. In support of this proposition suggests the high reactivity of terpenoid and aromatic compounds, which are the main components of the essential oils.

Pure essential oils are obtained by steam distillation, extraction by fats or other solvents. The choice of indicators of quality of essential oils depends on the application and is determined by their natural, pharmacological and taste-aromatic properties [2].

There are notworthy results obtained by the authors from the study of the essential oil composition of three samples of common yarrow plant *Achillea millefolium* affected by two circles empirically defined – the Spiral and the Antispiral, each of them grows the best in one of the eight directions. The conclusion is that the composition of essential oil from the three samples of common yarrow is not identical regarding the qualitative and quantitative composition of 83 components that are being studied. For example, the quantity of chamazulene in the control sample is 5.41 %, in the Spiral sample – 4.32 %, and in the Antispiral sample – 10.25 %. It can be concluded that the common yarrow plants grown in the Antispiral circle should be expected to have a stronger anti-inflammatory effect than those grown in the control group and the Spiral. The authors' main conclusion is that the primary principle of this study could be used for beautification of residential areas, for planting therapeutic parks, manufacturing herbal products, and many other activities related to using plants for human well-being and health.

Previously, we studied natural mineral water samples and cactus juice with the methods of spectral analysis of water – the NES and the DNES in order to evaluate the conditions for origin of life and living matter in hot mineral water [3–5], as well as we carrid out the moodeling of possible processes for origin of life and living matter in hot mineral water with deuterium [6–7]. These methods have proven themselves in a variety of biophysical studies of aqueous solutions, vegetable juices and extracts of plants. This has contributed to the promotion of the NES and the DNES in different biophysical research, including the study of the structure of water [8]. The research conducted by us demonstrated the role of water, its structure, the isotopic composition and physical-chemical properties (pH, temperature) on the growth and proliferation of prokaryotes and eukaryotes in water with different isotopic content [9–11]. These factors, the structure and composition of water are of great importance in many biophysical studies. The peculiarities of the chemical structure of the H_2O molecule and weak bonds caused by electrostatic forces and donor-acceptor interaction between hydrogen and oxygen atoms in H_2O molecules create favorable conditions for formation of directed intermolecular hydrogen bonds (O–H…O) with neighboring H_2O molecules, binding them into complex intermolecular associates which composition represented by general formula $(H_2O)_n$, where *n* can vary from 3 to 50 [12]. The hydrogen bond is a form of association between the electronegative O-atom and an H-atom, covalently bound to another electronegative O-atom, is of vital importance in the chemistry of intermolecular interactions, based on weak electrostatic forces and donor-acceptor interactions with charge-transfer. It results from interaction between electron-deficient H-atom of one H_2O molecule (hydrogen donor) and unshared electron pair of an electronegative O-atom (hydrogen acceptor) on

the neighboring H_2O molecule. By measuring the average energy among H_2O molecules in water samples by the NES- and DNES-methods it is possible to drow a conclusion about a number of hydrogen bonds in the sample and the distribution of individual H_2O molecules according to their energies. The method can also give information about the possible number of hydrogen bonds in water associates consisting of O–H…O–H groups and the distribution of H_2O molecules on the energy of the hydrogen bonds ($-E_{value}$) relative to the total energy of the hydrogen bonds ($E_{total\ value}$) in water samples [13]. For this purpose the model of W. Luck is used, improved by the authors, which consider water as an associated liquid, consisted of O–H…O–H groups [14]. The major part of these groups is designated by the energy of hydrogen bonds ($-E$), while the others are free ($E = 0$). The energy distribution function $f(E)$ is measured in reversed electron-volts (eV^{-1}) and may be varied under the influence of various external factors on water as temperature and pressure. The difference $\Delta f(E) = f(E_{\text{samples of water}}) - f(E_{\text{control sample of water}})$ – is designated the "differential non-equilibrium energy spectrum of water" (DNES). The DNES is a measure of changes in the structure of water as a result of external factors, because the energy of hydrogen bonds in water samples differ due to the different number of hydrogen bonds in water samples, which may result from the fact that different water samples have different structures and composition and various intermolecular interactions – the various associative elements etc. The redistribution of H_2O molecules in water samples according to the energy is a statistical process of dynamics. By using this method we calculated the average energy of the hydrogen bonds ($\Delta E_{H...O}$) between the H_2O molecules in water samples, which is $\Delta E_{H...O}$ = -0.1067 ± 0.0011 eV. This method was successfully applied by us earlier to the study of various water samples, e.g. of human blood serum, juice plants, as well as electro-chemically activated water solutions of catolite and anolyte [15] and water after the interaction with the natural minerals – zeolite and schungite [16]. As a result of these studies was evaluated a mathematical model of the interaction of these minerals with water, based on the change in the energy of the hydrogen bonds between H_2O molecules, with a regularity of change of energy of hydrogen bonds between H_2O molecules in the process of water treatment by shungite and zeolite. Natural waters derived from various Bulgarian water springs as well as water with varying deuterium content and the human blood serum of cancer patients were investigated by NES and DNES methods as well [17, 18]. As estimation factor was measured the values of the average energy of hydrogen bonds ($\Delta E_{H...O}$) among H_2O molecules, as well as local maxima in DNES-spectra of various samples of water and human blood serum at $\Delta E_{H...O}$ = -0,1387 eV. It was found that for a group of people in critical condition of life and patients with malignant tumors the greatest values of local maxima in DNES-spectra are shifted to lower energies relative to the control group. As a result we demonstrated a regularity of change of energy of hydrogen bonds between H_2O molecules in the various samples. The results also suggest the restructuring of the energy values among the individual H_2O molecules with a statistically reliable increase of local maximums in DNES-spectra. As a result, it was constructed a general mathematical model of water, based on the consistent patterns of change of hydrogen bonds between H_2O molecules and their distribution according to energies, which has been applied in many other studies of various samples of water, including mineral, water, mountain water, melt water and the electro-activated water. The level of reliability of the results obtained by the DNES-method according to the Student's *t*-test compiles < 0.05, which makes this method as a reliable method in various biophysical studies.

The main objective of the study was to gather scientific information and to analyze the data obtained through the application of the Dance of the Spiral methodology to the people who suffer from various ailments and to the composition of essential oils of plants planted according to this methodology. The results were analyzed using various scientific methods as NMR, gas chromatography, gas chromatography-mass spectrometry, NES, and DNES.

2. Material and Methods

2.1. Objects of Study

The main objects of study were common yarrow *(Achillea millefolium*), wood violet *(Viola odorata)*, dandelion *(Taraxacum officinale complex)*, common chicory *(Cichorium intybus)*, shepherd's purse *(Capsella bursa-pastoris)*, cranesbill *(Geranium macrorrhizum)*, broadleaf plantain *(Plantago major)*, and snowdrop *(Galanthus nivalis)*. The Spiral and the Antispiral

circles, created according to the Dance of the Spiral methodology, with plants positioned in one of the eight directions according to the empirical law established by the authors. All plants were planted and cultivated under the same conditions on the same plots of land with the same composition of the soil, the same lighting and irrigation. They were harvested after 3 weeks under the same condition, isolated and analyzed under the same procedure.

2.2. Clinical Trials with Volunteers

Clinical trials with 20 volunteers who suffer from neurological, gastro-intestinal, cardio-vascular, and articular conditions and diseases, who stayed consecutively in the Antispiral and Spiral plant circles for 6 min. in each circle were performed based on their subjective assessment of their state of health and their experience after the procedure of being stayed in the Antispiral and Spiral plant circles. These clinical trials resulted in the conclusion that the Antispiral circle has a relaxing effect while the Spiral circle has a stimulating effect upon tested people.

2.3. Study the Essential Oil Composition of Plants

The essential oil composition of the three samples has been studied designated as: "Regular", "Spiral", and "Antispiral". The essential oils were obtained and isolated under identical experimental conditions from air dry over ground parts by micro distillation-extraction in Likens-Nickerson device [19] for simultaneous distillation extraction. The further analysis was performed by using the standard methods of gas chromatography and gas chromatography-mass spectrometry. To determine the similarities in the essential oil composition of the three samples the Principal Component Analysis (PCA) has been performed. The analysis was carried out at the Bulgarian Academy of Sciences.

2.4. NES and DNES Spectral Analysis

The device for the DNES spectral analysis was made by A. Antonov on an optical principle. For this was used a hermetic camera for evaporation of water drops under room temperature (+22–24 ^{o}C) conditions. The water drops were placed on a water-proof transparent pad, which consists of thin maylar folio and a glass plate. The light was monochromatic with filter for yellow color with wavelength at = 580±7 nm. The device measures the angle of evaporation of water drops from 72.3^{o} to 0^{o}. The DNES-spectrum was measured in the range of E = -0.08– -0.1387 eV or λ = 8.9–13.8 μm using a specially designed computer program. The main estimation criterion was the average energy ($\Delta E_{H...O}$) of hydrogen O...H-bonds between H_2O molecules in water samples. The following samples were studied: water extracts from the common yarrow (*Achillea millefolium*) from the control group grown in the plant's natural habitat, and from the plants grown correspondingly in the Spiral and Antispiral circles. The water extracts were prepared according to the standard method authored by the landscape architect P. Penkov. Stems were not being removed but put for 8 min into prepared beforehand 330 ml glass bottles, filled with deionized water. The bottles are being closed, wrapped into aluminum foil, and labeled. Prior to biophysical study the water extracts were kept in the glass bottles for 24 hours at t = +4 ^{o}C.

2.5. Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy

The NMR spectroscopy was used as a method for quantitative analysis of 83 components of common yarrow *(Achillea millefolium*) on a device NMR spectroscopy on a Brucker WM-250 ("Brucker Daltonics" Germany) with a working frequency 70 MHz (internal standard – Me_4Si).

3. Results and Discussion

During the experiment the eight plants are planted into two circles. In the first circle, the Spiral, 1 m in diameter, they are planted in the following directions: north, northeast, east, southeast, south, southwest, west, northwest. D. Krastev, enters the empirical elements linked to the directions, i.e. sky, water, mountain, wood/wind, fire, lake, earth, thunder [20]. The plants used in the research: common yarrow *(Achillea millefolium*), wood violet *(Viola odorata)*, dandelion *(Taraxacum officinale complex)*, common chicory *(Cichorium intybus)*, shepherd's purse *(Capsella bursa-pastoris)*, cranesbill *(Geranium macrorrhizum)*, broadleaf plantain *(Plantago major)*, and snowdrop *(Galanthus nivalis)*, were positioned in circles by directions

(north, northeast, east, southeast, south, southwest, west, northwest) and elements (sky, water, mountain, wood/wind, fire, earth, thunder) (see Table 1).

Table 1. Arrangement of plants by directions of the world in the Spiral circle

Direction	Element	Plant
North	Sky	Common yarrow (*Achillea millefolium L.*)
Northeast	Water	Wood violet (*Viola odorata L.*)
East	Mountain	Dandelion (*Taraxacum officinale complex*)
Southeast	Wood/wind	Common chicory (*Cichorium intybus L.*)
South	Fire	Shepherd's purse (*Capsella bursa-pastoris L.*)
Southwest	Lake	Cranesbill (*Geranium macrorrhizum L.*)
West	Earth	Broadleaf plantain (*Plantago major L.*)
Northwest	Thunder	Snowdrop (*Galanthus nivalis L.*)

Next to the Spiral circle there is the Antispiral, in which the plants are positioned mirroring the arrangement in the Spiral circle (See Table 2).

Table 2. Arrangement of plants by directions of the world in the Antispiral circle

Direction	Element	Plant
North	Sky	Shepherd's purse (*Capsella bursa-pastoris L.*)
Northeast	Water	Cranesbill (*Geranium macrorrhizum L.*)
East	Mountain	Broadleaf plantain (*Plantago major L.*)
Southeast	Wood/wind	Snowdrop (*Galanthus nivalis L.*)
South	Fire	Common yarrow (*Achillea millefolium L.*)
Southwest	Lake	Wood violet (*Viola odorata L.*)
West	Earth	Dandelion (*Taraxacum officinale complex*)
Northwest	Thunder	Common chicory (*Cichorium intybus L.*)

For the duration of two weeks clinical trials were conducted with 20 volunteers – men and women suffering from neurological, articular, gastro-intestinal and cardio vascular diseases. The volunteers stayed in the Spiral and Antispiral circles, 6 min. consecutively in each circle. It was determined that the experiences were varied for each volunteer.

Further medical consultations with volunteers, based on their subjective assessment of their state of health after the procedure of being stayed in the Antispiral and Spiral plant circles, resulted in the conclusion that the Antispiral circle has a relaxing effect while the Spiral circle has a stimulating effect.

In subsequent biophysical studies water extracts from common yarrow grown in the Spiral, the Antispiral, and the control group have been studied with the use of NES and DNES methods. The results obtained with the NES method were recalculated with the DNES method.

For the control group the result with the NES method is E = -0.1095 eV. This is the average result for the energy of hydrogen bonds between H_2O molecules in pure water samples.

The Spiral sample results are the following: with the NES method – E = -0.1136eV. The DNES is defined as the difference between the sample and the control sample; it is ΔE = (-0.1136) – (0.1095) = -4.1 meV. The result is statistically reliable and lies within the interval [-1.1 --- 1.1 meV]. It points to restructuring of H_2O molecules towards higher energies of hydrogen bonds within the interval (-0.08) – (0.14) eV. The effect had been reported by patients as stimulating.

The Antispiral sample results are: with the NES method – E = -0.1055 eV. The DNES is defined as the difference between the sample and the control sample: ΔE = (-0.1055) – (0.1095) = 4.0 meV; this result is statistically reliable and lies within the interval [-1.1 --- 1.1 meV]. It points to restructuring of H_2O molecules towards lower energies of hydrogen bonds within the interval (-0.08) – (0.14) eV. The effect had been reported by patients as relaxing.

Further a research was done into the essential oil composition of three samples of common yarrow oil. The common yarrow (*Achillea millefolium*) belongs to the family *Asteracea*, whose

taxonomical characteristic is essential oil, which is confirmed by modern scientific research. For instance, according to A. Konakchiev [21], "the results of the study demonstrate that with the exception of few samples, the representatives of the *Millefollium* group produce chamazulene."

The common yarrow essential oil contains basically sesquiterpenes formed from 3 isoprene units $(C_5H_8)_3$. According to the authors' data the chemical composition of the common yarrow is on 0.2–0.8 % essential oil, sesquiterpene lactone matricin, flavonoid glycosides, pyridone alkaloids, cyanogenic glycosides, tannins, phytosterols, vitamin C and vitamin K, manganese salts, etc. According to F. Candan [22], the chemical analysis of common yarrow reveals the presence of essential oil, tannins, flavonoids, sesquiterpene lactones, alkamyds, inulin, and vitamin C. The essential oil from the common yarrow is obtained by distillation with water vapors. Depending on the subspecies of the plant and on the process of distillation the oil fraction may be blue, green or brown. Blue-green color is due to the primary component of the oil – chamazulene, obtained from the lactone matricin during the distillation. The oil also contains sesquiterpene α–cariophelene. In addition, the essential oil contains sesquiterpene α-caryophyllene, mono- and bicyclic terpenes, cineol, α-pinene, δ-pinene, thujone, borneol, and camphor. According to literature data, common yarrow has anti-inflammatory property (*in vitro* – inhibition of human neutrophilic elastase, protease, pointing to additional mechanisms of the anti-inflammatory property of extracts and fractions of common yarrow), especially for inflammation of the digestive and female reproductive systems [23] spasmolytic property [24], styptic, sedative, tonic, spasmolytic, antipyretic, antimycotic properties [25] and may be applied to treating wounds. There have been reports that common yarrow essential oil has an antioxidant and antimicrobial effect *in vitro* against *Streptococcus pneumoniae*, *Clostridium perfringens*, *Candida albicans*, *Mycobacterium smegmatis*, *Acinetobacter woffii* and *Candida krusei*. According to the authors' data, chamazulene, contained in common yarrow essential oil, is responsible for its anti-inflammatory effect.

In our experiments with studying the composition of essential plant oil produced under different experimental conditions the first sample of common yarrow oil was the control sample; the second sample has been acted upon by the Spiral and the third sample – by the Antispiral circle. 83 components are registered in the three samples, in amounts above 0.1 % – in the control, Spiral, and Antispiral sample comprising, correspondingly, 98.03, 95.19, and 95.97 % of the total essential oil content (see Table 3). The components are identified by their retention times and mass spectral data, and compared with literature sources. It appears that the components by the following numbers (in Table 3) – 2, 4, 5, 10, 11, 12, 14, 17, 21, 22, 24, 25, 28, 30, 31, 32, 38, 42, 44, 49, 52, 58, 62, 63, 64, 65, 69, 71, 72, 73, 74, 78, 79, 82, 83 – are present in all three samples. Components 2, 6, 7 are present only in the Antispiral sample; components 19, 32, 45, 51, 53, 81, 82, 83 are present only in the Spiral sample; components 1, 7, 8, 9, 18, 19, 35, 40, 41, 46, 47,48, 56, 57 are present only in the control group; components 3, 14, 16, 23, 29, 39 are present only in the control group and Antispiral sample; components 36, 43, 54, 70, 75, 77 are present only in the control group and Spiral sample; components 25, 33, 50, 60, 61, 66, 67, 68, 80 are present only in Spiral and Antispiral samples. The experimental data suggests that essential oil in the three samples is not identical in its quantitative and qualitative composition of the 83 parameters that being studied. The samples differ both in the types of components and in their amounts (see Table 3). The largest is the amount of identical components in the three samples. The common yarrow essential oil is known for its anti-inflammatory and styptic properties evidently due to the presence of chamazulene in essential oil. Table 3 shows that the amount of chamazulene in the control sample is 5.41 %, in the Spiral sample – 4.32 %, and in the Antispiral sample – 10.25 %. The common yarrow grown in the Antispiral circle, therefore, should be expected to have a stronger anti-inflammatory effect than the specimens of this plant grown in the control and Spiral groups. The main conclusion that can be drawn from the study of the essential oil composition is that the three samples of the common yarrow may have various pharmacological effects on tested people; however, this conclusion needs to be proven by future research in this area.

Table 3. Common yarrow essential oil composition of the three samples: Control, Spiral, and Antispiral

№	Components	Control	Spiral	Antispiral
1	santolina triene	0.97	–	–
2	thujene	–	–	0.36
3	pinene	1.85	0.53	3.06
4	camphene	0.39	–	0.70
5	sabinene	1.88	0.31	1.24
6	pinene	8.31	1.97	9.56
7	myrcene	–	–	0.38
8	2-dehydrocineole	0.28	–	0.24
9	yomogi alcohol	0.47	–	–
10	terpinene	0.22	–	–
11	p-cymene	0.27	0.22	0.32
12	limonene	0.33	0.23	0.53
13	1,8-cineole	8.17	5.02	11.15
14	*cis*-ocimene	0.17	–	0.36
15	salicyl aldehyde	0.13	0.27	0.19
16	terpinene	0.47	–	0.24
17	*cis*-sabinene hydrate	1.48	1.07	0.58
18	artemisia alcohol	0.26	–	–
19	terpinolene	0.16	–	–
20	linalool		0.39	–
21	*trans*-sabinene hydrate	0.46	0.82	0.58
22	campholene aldehyde	0.22	0.78	0.27
23	nopinone	0.21	–	0.25
24	*trans*-pinocarveol	0.77	1.37	0.25
25	camphor	1.97	9.25	17.20
26	M=152 $M_{10}H_{16}O$	–	0.60	0.38
27	*trans*-chrysanthemol	9.01	–	0.32
28	*cis*-chrysanthenol	1.66	1.16	2.00
29	M=152 $C_{10}H_{16}O$	2.31	–	0.85
30	borneol	14.29	10.75	3.62
31	terpinene-4-ol	1.74	0.77	1.03
32	terpineol	3.52	3.64	2.39
33	myrtenol	–	0.65	0.25
34	myrtenal	1.22	–	0.22
35	*trans*-carveol	0.30	–	–
36	*iso*-geraniol	0.37	0.57	–
37	*cis*-chrysanthenyl acetate	0.44	7.34	0.42
38	*trans*-chrysanthemyl acetate	4.18	0.36	1.53
39	lavandulyl acetate	0.48	–	0.18
40	non identificated	0.34	–	–
41	*trans*-carveyl acetate	0.41	–	–
42	terpinyl acetate	0.80	0.96	0.61
43	copaene	0.27	0.46	–
44	bourbonene	0.39	1.08	0.89
45	elemene	–	1.18	–
46	*cis*-jasmone	0.32	–	–
47	M-150 $M_{10}H_{14}O$	0.22	–	–
48	C_{10}-butanoate	0.20	–	–
49	caryophyllene	4.15	4.40	3.82
50	copaene	–	0.25	0.18

51	Z-farnesene	–	0.19	–
52	humulene	0.72	0.74	0.61
53	a-muurolene	–	0.19	–
54	ar-curcumene	0.17	0.19	–
55	germacrene D	2.29	8.89	6.91
56	a-zingiberene	0.21	–	–
57	M-238	0.32	–	–
58	indipone	0.24	0.92	0.49
59	bicyclogermacrene	0.42	1.81	0.72
60	a-cadinene		0.43	0.29
61	nerolidol	0.37	1.10	0.44
62	isocaryophyllene epoxide A	–	0.35	0.12
63	caryophylla-4(12),8 (13)-dien-5-one	0.35	0.60	0.27
64	spathulenol	0.52	1.12	0.54
65	caryophyllene oxide	4.15	5.88	3.91
66	salvial-4(14)-en-1-one	–	0.51	0.26
67	3Z-caryophylla-3,8(13)-dien-5-one	–	1.02	0.52
68	M=220 $C_{15}H_{24}O$	–	0.38	0.25
69	M=220 $C_{15}H_{24}O$	0.22	0.35	0.25
70	M=220 $C_{15}H_{24}O$	–	0.49	–
71	M=220 $C_{15}H_{24}O$	0.24	0.25	0.41
72	*cis*-cadin-4-en-7-ol	4.13	1.53	0.59
73	caryophylla-4(12),8 (13)-dien-5-ol	0.69	1.49	0.50
74	M=220 $C_{15}H_{24}O$	0.19	0.32	0.27
75	M=222 $C_{15}H_{26}O$	0.31	0.61	–
76	M=218 $C_{15}H_{22}O$	0.36	0.95	0.27
77	M=220 $C_{15}H_{24}O$	0.22	0.46	–
78	M=220 $C_{15}H_{24}O$	0.88	2.40	1.33
79	M=220 $C_{15}H_{24}O$	0.56	1.37	0.21
80	CH-alifate carbon	–	0.32	0.41
81	M=218 $C_{15}H_{22}O$	–	0.39	–
82	M=220 $C_{15}H_{24}O$	–	0.22	–
83	chamazulene	5.41	4.32	10.25
Total		98.03	95.19	95.97

Notes:
*The results obtained from the research of the Bulgarian Academy of Science
**The table includes components which amounts in the oil exceed 0.1 %.

The results of this research have been compared with those of the study conducted in 1999 and 2000 on twenty samples of common yarrow inflorescences and leaves gathered in their eleven habitats in Eastern Lithuania [26]. The essential oils were analyzed using spectroscopic methods. According to the primary component of the essential oils, the samples have been divided into six chemotypes: pinene (10 samples, 10.2–17.2 %), 1.8 cineol (3 samples, 8.8–9.9 %), borneol (3 samples, 11.5–13.2 %), camphor (1 sample, 13.1 %), nerolidol (2 samples, 8.5–9.3 %), and chamazulene (1 sample, 20.1 %). These primary components are also present, in different consentrations, in the samples that we have studied (Table 3). Other experiments have established that eight of the studied samples do not contain chamazulene, and 1 sample contains only traces of this component. The researchers associate the curative power of the common yarrow and its essential oil with chamazulene. According to seven authors quoted in the above paper [26], the components – 1,8-cineol, camphor, borneol, nerolidol, caryophyllene, and caryophyllene oxide – display different biological activity, which agrees with our conclusion that the three samples of common yarrow, depending on their phytochemical composition, have different pharmacological effects. These results are broadly in line with our results.

Conclusions

1. The results obtained by means of biophysical methods for measuring the energy of the hydrogen bonds among H_2O molecules – Non-equilibrium energy spectrum (NES) and Differential non-equilibrium energy spectrum (DNES) – in the samples of water extracts of common yarrow (*Achillea millefolium*) from the control group growing in the plant's natural habitat, and from the plants cultivated correspondingly in the Spiral and the Antispiral circles are harmonic in their absolute value and demonstrate the uniqueness of the Dance of the Spiral methodology (Krastev, 2011). The effect of the common yarrow plants from the Spiral circle is stimulating, while the effect of those from the Antispiral circle is relaxing.

2. The results of the studies of the essential oil composition from the three samples of common yarrow from the three groups – control, Spiral, and Antispiral – lead to a remarkable conclusion: the essential oils from the three samples are not identical in the quantitative and qualitative composition regarding the 83 components detected in them and studied, i.e. the essential oils from the three samples of common yarrow differ regarding their components and the amounts of these components. The amount of chamazulene was found to be equal to: in the control group sample – 5.41 %; in the Spiral sample – 4.32 %; in the Antispiral sample – 10.25 %; i.e. the amount of chamazulene in the essential oil from the Antispiral sample is almost twice of that in the control group and Spiral samples. It follows that the common yarrow cultivated in the Antispiral circle should be expected to have a stronger anti-inflammatory effect than the common yarrow from the control group and the Spiral sample.

3. Pharmacognostical and biophysical methods for measuring the energy of hydrogen bonds between H_2O molecules in the water extracts of common yarrow cultivated in the Spiral and Antispiral circles pointed out to the curative effects of these plants.

4. The authors' summarizing conclusion is that the principles established by their research can be implemented in landscaping and beautification projects, planting parks that have health-restoring effects, manufacturing herbal products, and in many areas related to cultivation of plants and to the health and well-being of people.

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References

1. Asenov I.C., Gusev G., Kitanov N., Nikolov S., Petkov T. *Herb Gathering*. – Biler: Sofia, 1998 [in Bulgarian].

2. Petkov V. *Modern Phytotherapy*. – Sofia: Bulgaria. 1982 [in Bulgarian].

3. Ignatov I. Water in the Human Body is Information Bearer about Longevity / in: *Conference on the Physics, Chemistry and Biology of Water*. – NY: Vermont Photonics, 2012.

4. Ignatov I., Mosin O.V. Methods for Measurements of Water Spectrum. Differential Non-equilibrium Energy Spectrum Method (DNES) // *Journal of Health, Medicine and Nursing***.** 2014. Vol. 6. P. 50–72.

5. Ignatov I., Mosin O.V., Velikov B. Longevity Factors and Mountain Water of Bulgaria in Factorial Research of Longevity // *Journal of Medicine, Physiology, Biophysics*. 2014. Vol. 1. P. 13–33.

6. Ignatov I., Mosin O.V. Possible Processes for Origin of Life and Living Matter with Modeling of Physiological Processes of Bacterium *Bacillus subtilis* in Heavy Water as Model System // *Journal of Natural Sciences Research*. 2013. Vol. 3(9). P. 65–76.

7. Ignatov I., Mosin O.V. Modeling of Possible Processes for Origin of Life and Living Matter in Hot Mineral and Seawater with Deuterium // *Journal of Environment and Earth Science*. 2013. Vol. 3(14). P. 103–118.

8. Ignatov I., Mosin O.V., Velikov B. Mountain Water as a Factor of Human Longevity. Local Extremum at 8.95 μm in Spectrum of Water as Indicator for Health and Longevity // *Journal of Medicine, Physiology and Biophysics*. 2015. Vol. 9. P. 51–81.

9. Mosin O., Ignatov I., Skladnev D., Shvets V. Studying of Phenomenon of Biological Adaptation to Heavy Water // *European Journal of Molecular Biotechnology*. 2014. Vol. 6. № 4. P. 180–209.

10. Mosin O., Ignatov I. Biological Adaptation of Organisms in Heavy Water // *Journal of Health, Medicine and Nursing*. 2014. Vol. 7. P. 101–140**.**

11. Mosin O., Ignatov I. The Biological Adaptation to Deurerium Oxide. Phenotypic or Genotypic Phenomenon? // *Journal of Medicine, Physiology and Biophysics*. 2015. Vol. 16. P. 12–28.

12. Ignatov I., Mosin, O.V. Structural Mathematical Models Describing Water Clusters // *Journal of Mathematical Theory and Modeling*. 2013. Vol. 3(11). P. 72–87.

13. Ignatov I., Mosin O.V. Structural Models of Water and Ice Regarding the Energy of Hydrogen Bonding // *Nanotechnology Research and Practice*. 2015. Vol. 7. № 3. P. 96–118.

14. Ignatov I., Mosin O.V. Methods for Research of Mountain and Melt Water as Factor of Longevity. Chemical Composition, NES and DNES Methods for Spectral Analysis. Effects of Calcium, Magnesium, Zinc and Manganese // *Advances in Physics Theories and Applications*. 2015. Vol. 44. P. 48–64.

15. Gluhchev G., Ignatov I., Karadzhov S., Miloshev G., Ivanov I., Mosin O.V. Studying of Virucidal and Biocidal Effects of Electrochemically Activated Anolyte and Catholyte Types of Water on Classical Swine Fever Virus (CSF) and Bacterium *E. coli DH5* // *Journal of Medicine, Physiology and Biophysics*. 2015. Vol. 13. P. 1–17.

16. Ignatov I., Mosin O.V. The Structure and Composition of Carbonaceous Fullerene Containing Mineral Shungite and Microporous Crystalline Aluminosilicate Mineral Zeolite. Mathematical Model of Interaction of Shungite and Zeolite with Water Molecules // *Advances in Physics Theories and Applications*. 2014. Vol. 28. P. 10–21.

17. Ignatov I., Mosin O.V., Bauer E. Vortex Power Spring Water: Physical-Chemical Qualities of this Water compared to Mountain and Melt Water from Bulgaria, Russia and Glacier Rosenlaui from Swiss Alps // *Advances in Physics Theories and Applications*. 2015. Vol. 45. P. 6–29 .

18. Ignatov I., Mosin O.V., Velikov B., Bauer E., Tyminski G. Research of Longevity Factors and Mountain Water as a Factor in Teteven Municipality, Bulgaria // *Journal of Medicine, Physiology and Biophysics*. 2014. Vol. 2. P. 37–52.

19. Nickerson G.B., Likens S.T. Gas chromatography evidence for the occurrence of hop oil components in beer // *J. Chromatogr*. 1966. Vol. 21(1). P. 1–5.

20. Krastev D. *The Dance of the Spiral*. Stara Zagora: Art Hemus, 2011. 167 p [in Bulgarian].

21. Konakchiev A. Essential Oils of the Varieties of *Lavandula Angustifolia Mill*, species of the genus *Achillea*. Organic Chemistry Institute, Phytochemistry Center, Bulgarian Academy of Sciences, Sofia, PhD Dissertation, 2015 [in Bulgarian].

22. Candan F., Unlu M., Tepe B., Daferera D., Polissiou M., Sokmen A., Akpulat H.A. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium subsp. millefolium Afan. (Asteraceae)* // *J. Ethnopharmacol*. 2003. Vol. 87(2–3). P. 215–220.

23. Ivancheva S., Nikolova M., Tsvetkova R. Pharmacological Activities and Biologically Active Compounds of Bulgarian Medicinal Plants / in: *Phytochemistry: Advances in Research Book Phytochemistry,* 2006. 87–103.

24. Benedek B., Kopp B. *Achillea Millefolium L. s.l.* Revisited: Resent Findings Confirm the Traditional Use // *Wien Med Wochehsher*. 2007. Vol. 157(13–14). P. 312–314.

25. Figueiredo A., Pais M.S., Scheffer J. *Achillea millefolium L. ssp. Millefolium* (Yarrow): *in vitro* Culture Production of Essential Oil / in: *Biotechnology in Agriculture Foresty*. – New York: Springer, 1995. Vol. 33. P. 1–20.

26. Moskute D., Judzenziene A. Chemotypes of the Essential Oils of *Achillea Millefolium L spp Millefolium* Growing Wild in Eastern Lithuania // *Chmija*. 2002. Vol. 13(3). P. 168–173.

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Исследование эффектов методологии "спирального танца" на физиологические параметры растений и содержание эфирных масел

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Аннотация. “Спиральный танец” – оригинальная методология, состоящая из физических упражнений на основе древней оздоровительной практики и долголетия. В данном исследовании эта методология была применена для растений. Использовались 8 растений – тысячелистник обыкновенный, фиалка душистая, одуванчик лекарственный, цикорий, пастушья сумка обыкновенная), герань крупнокорневищная, подорожник широколиственный и подснежник, каждый из которых лучше растет в одном из восьми направлений. Посадка была выполнена в восьми направлениях, так как каждое упражнение предположительно влияет на состояние здоровья. Два круга определялись эмпирически как спираль и антиспираль. Клинические испытания с 20 добровольцами страдающими от неврологических, желудочно-кишечных, сердечно-сосудистых и суставных заболеваний, которые находились последовательно в спиральном и антиспиральном кругах в течение 6 мин в каждом круге на основе их субъективной оценки их состояния здоровья и опыта определялись как расслабляющий эффект (антиспираль-круг) и стимулирующее действие (спираль-круг). Дальнейшие исследования включали изучение состава эфирных масел методами ^{1}H ЯМР, газовой хроматографии и хромато-масс-спектрометрии, а также спектрального анализа методами неравновесного энергетического спектра (НЭС) и дифференциального неравновесного энергетического спектра (ДНЭС) водных экстрактов из тысячелистника обыкновенного (контрольная группа, произрастающая в естественной среде обитания) и растений, выращенных соответственно в спиральном и антиспиральном кругах. В образце тысячелистника из спирального круга зафиксировано увеличение средней энергии водородных связей между молекулами H_2O. В образце тысячелистника из антиспиральной круга зафиксировано уменьшение средней энергии водородных связей между молекулами H_2O. Качественный и количественный состав 83 компонентов эфирных масел из образцов растений, выращенных в спиральном и антиспиральном круге, различные. Содержание хамазулена в контрольной группе – 5.41 %; в спиральном образце – 4.32 %; в антиспиральном образце – 10.25 %; т.е. количество хамазулена в эфирном масле из антиспирального образца почти в два раза превышает содержание хамазулена в образце контрольной группы и спиральном образце.

Ключевые слова: тысячелистник обыкновенный, эфирные масла, НЭС, ДНЭС, ^{1}H ЯМР.

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Ancient Paleo-DNA of Pre-Copper Age North-Eastern Europe: Establishing the Migration Traces of R1a1 Y-DNA Haplogroup

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Abstract

The work considers the problems of paleogenetics and anthropology connected with problem of pre-Copper Age after-Glacial repopulation process of the North-Eastern Europe. The unified data, obtained in various laboratories in 2010-2016, collects a certain amount of the ancient mt-DNA and Y-DNA haplogroup samples of the considered period, what allows establishing the connection between some of them, comparing them with the data of neighboring regions, and attributing them to certain migration flows traceable in archeology. The paper makes an attempt to build a picture of the population of North-Eastern Europe in pre-Copper Age time and to systemize the paleo DNA genotyping results into clusters corresponding to different migration waves. The paper can be of use for biomedical purposes also, as some correlations between diseases and haplogroups were noticed in various medical works.

Keywords: Y-DNA haplogroup, R1a1, J2b, mtDNA haplogroups U4 and U5a1, paleogenetics.

1. Introduction

The interest in the origin and early localization of carriers of Y-DNA haplogroup R1a1 is serious, since this Y-DNA subclade is inherent to the significant percentage of the population of Central and Eastern Europe, India, Middle East. It is widely recognized and already proven in terms of archeology and paleogenetics that a significant concentration of R1a1 Y-DNA haplogroup was inherent to the population of European Corded Ware culture (authors note that there also existed less famous East Asian Corded Ware culture group [1]). However, the location of R1a1 bearers of Pre-Corded Ware horizons causes debates due to a lack of experimental data. Nevertheless, over the last year such data emerged, allowing formulating a data-based hypothesis.

The domain of palogenetics is still young but rapidly developing. Much data from different archaeological cultures are obtained from different research groups. Among very profound and deep works the authors can mention [2-7] which give the wide picture of dispersion of mitochondrial and Y-chromosome haplogroups in prehistoric and early historical layers. This works offer a certain profound conclusions of some archaeological group origins (for example,

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a connection between Corded Ware and Yamnaya) but the conclusions deal with Copper Age and Bronze Age Cultures.

The main aim of the work is to make a certain conjecture based on available data on the distribution of Y-DNA haplogroup R1a1 in Pre-Copper Age Europe. In spite of the scarcity of findings, the rigor archaeological attribution of the findings allows tom make a certain conclusion which reconstructs the areal. The main data contains in works [5,6, 8, 9] Namely, the bearers of Neolithic R1a1 may have been concentrated in the area of Comb Ware Pottery, which precedes the Corded Ware cultures what are proven to be the areal of R1a1 dominance, and which comes from the East, as the Comb Ware pottery centers are situated in Easter European plain.

The serious importance of ancient haplogroup distribution knowledge is connected with possible correlations between haplogroups and different hereditary diseases. The ancient locations of haplogroups for which the correlations are established can point where the probability of a certain genetic illness is higher, and so the inhabitants of these areas should make genotyping more actively. For example in [10] the correlation between mtDNA haplogroups B5 and possibly V20 and Alzheimer disease probability was mentioned. This approach could also work for positive characteristics as longevity. For example, in popular and scientific literature the peoples of Hunza-Burushaski and Abkhazians considered to contain higher percentage of long-living people that most other populations. Though this information is disputable and should be verified, it should be mentioned, that both peoples belong to Sino-Caucasian language macrofamily and according to [11] the spread of the languages of that kind is associated with Y-DNA haplogroups Q and R. In [12] is pointed that the representatives of old subclades of R1a were find in Iran, Eastern Turkey and Caucasus. Also, Y-DNA haplogroup R2 (~14%), related to old R1 Y-haplogroup parental to R1a1 and a high percentage of R1a1 (~25%) are inherent to Burushaski [13]. According to [14] high percentage of R1a1 (~33%) is found among Abkhazians. So the hypothesis of corresponding of the higher longevity within the bearers of the old subclades of R and R1a compared to the newer (to which many European peoples belong) could be rigorously tested and if the hypothesis is true, the factors which yield such situations should be studied.

Also the paper can be useful for popular genetic specialists themselves as they can use it to decide which new archaeological sites to study to verify the outlined hypothesis, if they are interested in Y-DNA haplogroups R and J, mtDNA haplogroups U4 and U5.

2. Material and Methods

The main materials for the research are data from paleogenetic samples of Y-DNA haplogroups, described in other research works, namely [7, 10, 9, 12] grouped in Table 1.

All these results were obtained by the defining haplogroups of the ancient genomes by the genotyping procedure.

In [5] the insolution hybridization capture was used to enrich next generation sequencing libraries for a target set of 394,577 single nucleotide polymorphisms (SNPs) (“390k capture”), 354,212 of which were autosomal SNPs that have also been genotyped using the Affymetrix Human Origins array in 2,345 humans from 203 population.

In [8] the DNA was extracted by the CTAB method. To avoid the effects of degradation, the amplification of HVS1 mtDNA was conducted with the help of single round PCR represented by four overlapping amplicons.

In [6] in-solution hybridization was also used with synthesized oligonucleotide probes to enrich promising libraries for more than 1.2 million SNPs (“1240k capture”). The targeted sites included nearly all SNPs on the Affymetrix Human Origins and Illumina 610-Quad arrays, 49,711 SNPs on chromosome X and 32,681 on chromosome Y, and 47,384 SNPs with evidence of functional importance. To learn about the history of archaeological cultures for which genome-wide data was reported for the first time, were studied either 1,055,209 autosomal SNPs when analyzing 230 ancient individuals alone, or 592,169 SNPs when co-analyzing them with 2,345 present-day individuals genotyped on the Human Origins array.

In [9] to reduce the effects of post-indexing contamination, raw reads were retained if the Hamming distance for the observed index was within 1 base of the expected index. Adapter sequences were trimmed from the 3’ ends of reads using cutadapt version 1.3 (ref. 35), requiring an overlap of 1 bp between the adapter and the read. As ancient DNA damage is more apparent at the ends of sequences, the first and last two bp of all reads from the deep sequencing phase of analysis

were removed using SeqTK. A minimum read length of 30 bp was imposed. Sequences were aligned using Burrows-Wheeler Aligner (BWA) version 0.7, with the seed region disabled, to the GRCh37 build of the human genome with the mitochondrial sequence replaced by the revised Cambridge reference sequence (NCBI accession number NC_012920.1). Sequences from the same sample were merged using Picard MergeSamFiles and duplicate reads were removed using SAMtools version 0.1.19. Average depth of coverage was calculated using genome analysis toolkit (GATK) Depth of Coverage and indels were realigned using RealignerTargetCreator and IndelRealigner from the same suite of tools. Only data from the deep sequencing phase of the project (100 bp single-end sequencing on a HiSeq ,000) were used in the subsequent analyses.

The main research method of this paper is the interpretation of recently obtained genetic data, which is compared with archaeological cultures distribution. To support some conclusions, data on mtDNA haplogroups was also used as supplementary instrument.

Table 1. The main results of paleoDNA genotyping from the Eastern Europe archaeological sites

Sample	Y-DNA	MtDNA	Source
R1a1			
Yuzniy Oleni Ostrov burial № 125, 5500-5000 BCE.	R1a1 M459+, M198-	C1g (formerly C1f)	[5, p. 25]
Serteya archeological site, middle of V-IV mill. BCE	R1a1	H2	[8, p. 294]
Khvalynsk-II burial 5200-4000 BCE.	R1a1, preliminary classification was determined as R1a1 M459+, M198- [**15]**	U5a1i	[6]
J			
Yuzniy Oleni Ostrov burial № 40, 5500-5000 BCE.	J	U4a	[6]
Satsurblia burial (Georgia), Upper Paleolithic	J	K3	[9]
Kotias burial (Georgia), Mesolithic	J2a	H13c	[9]

3. Results and Discussions

R1a1*, M459+, M198- on Yuzhniy Oleni Ostrov burial (North-Western Russia)

One of the most well studied Eastern European archeological sites is the Yuzhniy Oleni Ostrov burial on the shores of Lake Onega (Karelia, Russia) and it is dated back to the developed and late Mesolithic period of VII-V millennium BC. Three individuals from the Yuzhniy Oleni Ostrov, who lived 7500 years ago (UZOO-7, 8 and UZOO-UZOO-74), possessed a non-existing now in Europe mitochondrial haplogroup C1f [2]. Also among the burials of Yuzhniy Oleni Ostrov were found mitochondrial haplogroups U4, U2e, U5a [3], J and H [4, p. 36]. The Mesolithic inhabitant of Yuzhniy Oleni Ostrov (burial № I0061) possessed Y-chromosome haplogroup R1a1 (SRY10831.2, M198- subclade) [5] and mitochondrial haplogroup C1g (formerly C1f) [5]. The other Mesolithic inhabitant of Yuzhniy Oleni Ostrov (I0221 / UZ0040) possessed Y-chromosome haplogroup J, and mitochondrial haplogroup U4 [6].

The author of one the most detailed publication on Yuzhniy Oleni Ostrov antropology V.P. Yakimov adhered to the Eurocentric point of view on the formation of the Mesolithic Onega inhabitants. He suggested that their origins are linked to the Paleolithic population of Eastern Europe, who moved along the glacier to the northern and northeastern directions [16]. But some

cross-breeding with Eastern population was also confirmed: «*Later, it was concluded that it belonged to the described in the southern edge of the region so-called «flint» Mesolithic culture associated by origin with cultures of the Volga-Oka area, and (since the appearance in the VIII millennium BCE) coexisted with an earlier (since the X millennium BCE) local «quartz-slate» culture created by people from the North Urals and Trans-Urals and related to Finnish Askola – Suomusyarvi*» [17]. But as we see further, ultimate western origins of that culture is probable also as we see a wide migration towards east in early Mesolithic. Currently, researchers emphasize that «*Yuzhniy Oleni Ostrov burial site is as an archaeological source extremely multifaced*» (ibid) and represents a particular genetic type, different from the classical Mongoloid and Caucasoid (ibid). A very heterogeneous composition of the population is now well proven by the presence of Y-chromosome haplogroup J, which indicates the influence of the southern areas and communication of Yuzhniy Oleni Ostrov people with populations of the Black and Caspian Sea.

The question if the culture of Yuzhniy Oleni Ostrov is non-ceramical Mesolithic or ceramical Neolithic, is still open. It should be mentioned that P.N. Tretiakov in his book «Finno-Ugrians, Balts and Slavs at the Dnieper and Volga» mentioned: «*The population, which left behind the Oleni Ostrov burial ground seems to be familiar with the ware. It is proven by the bone plates with pinked edge, which must have served as ornament molds for clayware. If this statement is true, the Oleni Ostrov burial ground should be connected with the ancient culture of the Pottery Neolithic of the East Baltics – the Sperrings culture»* and further: *«Comb ware of Sperrings type, according to different researchers, has the closest analogs to the Neolithic cultures of the Kama Region, the Cisuralian area and Trans-Urals, and we agree with this statement. Anyhow, there is no doubt that numerous Ural and Kama analogs to Sperrings ware, which have been detected in the recent years are incomparably more convincing than the Middle Dnieper one*» [18, p. 24]. This supports the version of the possible connections of Yuzhniy Oleini Ostrov people with the southern or eastern Neolithic cultures.

In addition to the local component the cultural influences on Yuzhniy Oleni Ostrov, the influences of most far-off regions have been mentioned in different works. For example, one article highlights the unexpected similarities of Yuzhniy Oleni Ostrov inhabitants and the representatives of culture Çatalhöyük [19, p. 92]: «*However, the distribution observed on the charts provokes a number of questions because of the convergence of typological characteristics of the groups diametrically opposed geographically and for which the likelihood of direct biological kinship and mutual contacts excluded. The most vivid illustration of this is the convergence of characteristics a series of Mesolithic Oleni Ostrov burial ground with sample from Çatalhöyük by the values of the second factor …*». But the finding of the Y-DNA haplogroup J, which is associated with significantly more southern regions, only confirms ties of Yuzhniy Oleni Ostrov with the Southern cultures.

This way, the southern Neolithic influences on Yuzhniy Oleni Ostrov seem to be strongly probable and they could be originated from the Neolithic tribes of Comb Ware cultures.

R1a1 in Serteya Culture (Western Russian Plain)

The Usvyaty Culture or the Usvyaty level of Serteya culture is another area of finding of an R1a1 bearer of pre-Corded Ware period [8, p. 294]. According to A.N. Mazurkevich: «*The first ceramic ware within Pskov area emerged no later than in the middle of the VI millennium BC. At this time the sites of Serteya culture were located in the Lovat-Dvina interfluve on the shores of lakes, running into the stream, connecting the basins…. The first clayware appeared. These are small raw clay vessels with cylinder body and conic bottom. The pots were made of the small clay 'stripes' with the beveled edge and were covered with the thin clay wash. After that the surface of the vessels was ornamented with the ornament mold, leaving the triangle, double or comb prints. The ornament was often molded in plotted manner. The similar ware emerged in forest, forest-steppe and steppe zones of the Russian Plain, as well as in the Lovat-Dvina interfluve. Other components of the material culture (stone, bone and horn artifacts) did not have the fundamental changes in time of the spread of this ware. It enables to propose the emergence of this type of ware in one center and the quick spread of the idea of the ware industry from this area. Such center was supposedly located in the Lower Volga Region and the North Caspian Sea Region in the VI millennium BC*» [**20**].

A.M. Miklyaev also gives a detailed picture of emerging the ware industry in Serteya Region «*The most ancient early Neolithic culture featuring ware development phases of the area is the Serteya Culture. This phase includes fragments of heavy-walled vessels produced through the 'overlap' method. After drying, the ribbons were jointed together and their joints were smoothed out by a comb press for a proper binding. The surface of ready vessels was covered with the thin clay wash and the ornament in a form of geometric composition, performed in a stroke-setback or (more rarely) in a stroke manner. The vessels were not burnt, but dried up. Judging by the ornament techniques (Smirnov, 1989), the idea for pottery manufacture came from Azov-Caspian Cultural province, but it cannot be proved by reliable sources of information so far. It should be noted that the Serteya Culture could have entered the Early Neolithic Community extending from the South of the Russian Plain to as far as Valday*». Therefore, the first stage has links to the Caspian region.

Then «*The next stage features the cauldron-type vessels. This time, the ornaments included more compositions performed by a comb press and the first appeared dents and cuts. As a rule, an ornament was placed in the upper part of a vessel. This stage ware has a narrower range of analogs – The Upper Dnieper tableware (Artemenko, 1954; Kalechits, 1987) and Lithuanian territory (Rimantane, 1966 and 1973). This may indicate to a separation of local groups within the above said community. In this case, it is a group located between two rivers: the Dvina and the Lovat, the Upper Dnieper and Lithuania. The links between the Upper Volga Region and the Left-bank Ukraine are getting weaker*» [21]. The Upper-Dnieper Culture located to the South of Serteya can be classified as a forest culture with comb ware traditions [22, p. 73] and could be considered as a more Western tradition than what had come from the North Caspian area and Lower Volga.

The genotyped carrier of R1a1 haplogroup belongs to Usvyaty culture group. A. M. Miklyaev indicates that Serteya culture can be divided into three phases: a, b and c, followed by phase d and e of Rudnya culture. Ceramic phase and is characterized by stroked technique, in phase b comb stamp appears, in the next phase c comb stamp becomes prevailing, holes and notches appear (A.M. Miklyaev with reference to Artemenko and Rimantene relates this last phase with Upper Dnieper Early Neolithic culture and Lithuanian Neolithic) [22, pp. 16-22]. Usvyaty culture dating from the late IV till the middle of the III millennium BC [23, p. 369] allows comparing its comb-stamped ceramics with a close-type comb-stamped pottery of the North European part of Russia, for example in Kargopol culture [24, p. 222]. In the A. M. Miklyaev's work is emphasized the connection of the population of the region with the Narva culture, especially during the Rudnya culture stage [21, p. 24]. In this respect, we can (of course, in the first schematic approximation) see in the history of ceramics in the region of Eastern Europe Neolithic period a certain competition between the two types of pottery: comb inherent to the North and the North –West and stroked, what came from the South and the South-East, and is most likely connected with the Lower Volga Neolithic cultures. And Usvyaty culture belongs to the time continuing the large period of comb ware domination, having undergone the influence of European Linear Band Ceramics.

So forth, the zone of Neolithic cultures of Serteya region was within the bigger zone of comb ceramics culture of Eastern Europe. During early stages, the eastern connections were prevailing, but then the west and south-west connection became defining. Usvyaty stage emerged after Comb Ware domination and moreover has definite Central European connections.

R1a1*- M459+, M198- in Khvalynsk-II burial (Steppe Volga Region)

The mentioned before connection between Serteya and the Low Volga Region lead us to the analysis of Khvalynsk-II burial, which also belongs to the pre-Corded Ware comb-stroke ornament group: «*Khvalynsk culture can be characterized by flat-bottomed and sharp bottomed ware … Ornament composed of the rows of horizontal strokes separated by horizontal wave lines, usually cowers all the ware specimen or its upper half*» [25, p. 39]. I.N. Vassilieva gives the characteristics to the ornaments of ware in the Khvalynsk I and Khvalynsk II burials: «*According to the opinion of I.N. Vassilieva, based on the microscopic research of the Khvalynsk ceramics technology, the ornament was made by the wicker factures… Sometimes the ornament was made by ammonite*

prints, strokes, short lines or comb stamp» [26, p. 66]. The similar technique has the analogs in the b and c phases of Serteya Culture, identified by A.M. Miklyaev [20].

The analysis of the ceramics of Khvalynsk culture shows that it definitely does not belong to the Corded Ware areal, and can be referred to as belonging to the cultures of comb-and-stroked pottery.

The areal of Comb Pottery cultures.
Y-haplogroup J2b as a possible companion of R1a1 on Neolithic sites

The analyzed material showed that the discovery of Y-haplogroup R1a1 bearers in pre-Corded Ware sites happens in the areas influenced by cultures of comb-stroked ceramic, and everywhere the presence of a comb ornament is noticeable. In the context of the analysis of cultural influences in the Eastern Europe, it is necessary to distinguish between stroked and comb pattern. Comb pattern is traditionally considered to be brought about from the north to the south of Eastern Europe, but D. L. Gaskevich in his long article «North Pontic Impresso: the origin of the Neolithic Pottery with Comb Decoration in the South Eastern Europe» [27] wrote the opposite. He made quite a bold assumption that runs counter to the tendency to minimize the migration, and proposed the origin of this type of pottery in the northern Black Sea coast.

«*The absolute data collected over the last 15 years in Kiev Radiocarbon Laboratory, have revealed that such ware appeared in the North-Pontic region earlier than in Upper Dnieper, Volga Region, Kama basin, Trans-Urals. However, in the steppe Pontic region it appeared earlier than in forest-steppe. All these data have proved unreliability of above mentioned hypothesis. As an alternative, the author suggests considering the Pontic region Neolithic area with comb ceramic ornamentation as a part of Neolithic cultures with Impresso ware from the Mediterranean region*» [27, p 246-247].

This way, according to D. Gaskevich we see in the Eastern Europe only a small episode of a big process, which took place from Sahara to Trans-Urals and from Marocco to the Levant. Probably, the early appearance of ceramics in Samara region and in the Mideterranian area are two faces of one wave of the spread of the Neolithic technologies. The ancient subclades of Y-DNA R1b haplogroup can be detected both in the Volga-Ural region and in the Northern Africa, and the Neolithic findings of R1b are attributed to the Elshanskaya (Lebyazhinka IV) and Els Trocs cave (Spain). At the moment of writing the paper the opinion about the spread of R1b Y-haplogroup carriers from the Eastern Eurasia is dominating, and in moving westward the R1b (and may be R1a) bearers of ceramic Neolithic technologies could obviously merge with the carriers of other haplogroups, J among others.

As D.L. Gaskevich refers to the initial spread of the Neolithic within the Eastern Europe, we should consider the issue of the genetic reflection of this process and specify the genetic map of the North Black Sea Region and the adjust territories in the period, preceding the Mediterranean ware adoption (though, there is another possible address of this initial spread, namely Elshanskaya culture and its derivates up to the Crimea). In VIII-VII millennia BC the North Black Sea Region was inhabited by the bearers of the Kukrek and Zimnikovskaya cultures [28, p. 44-45], the final Paleolithic includes Osokorovskaya culture, considered with the Caucasian Imereti one as a group of «Epi-Gravettian traditions» (ibid, [29, p. 43]) (Figure 1).

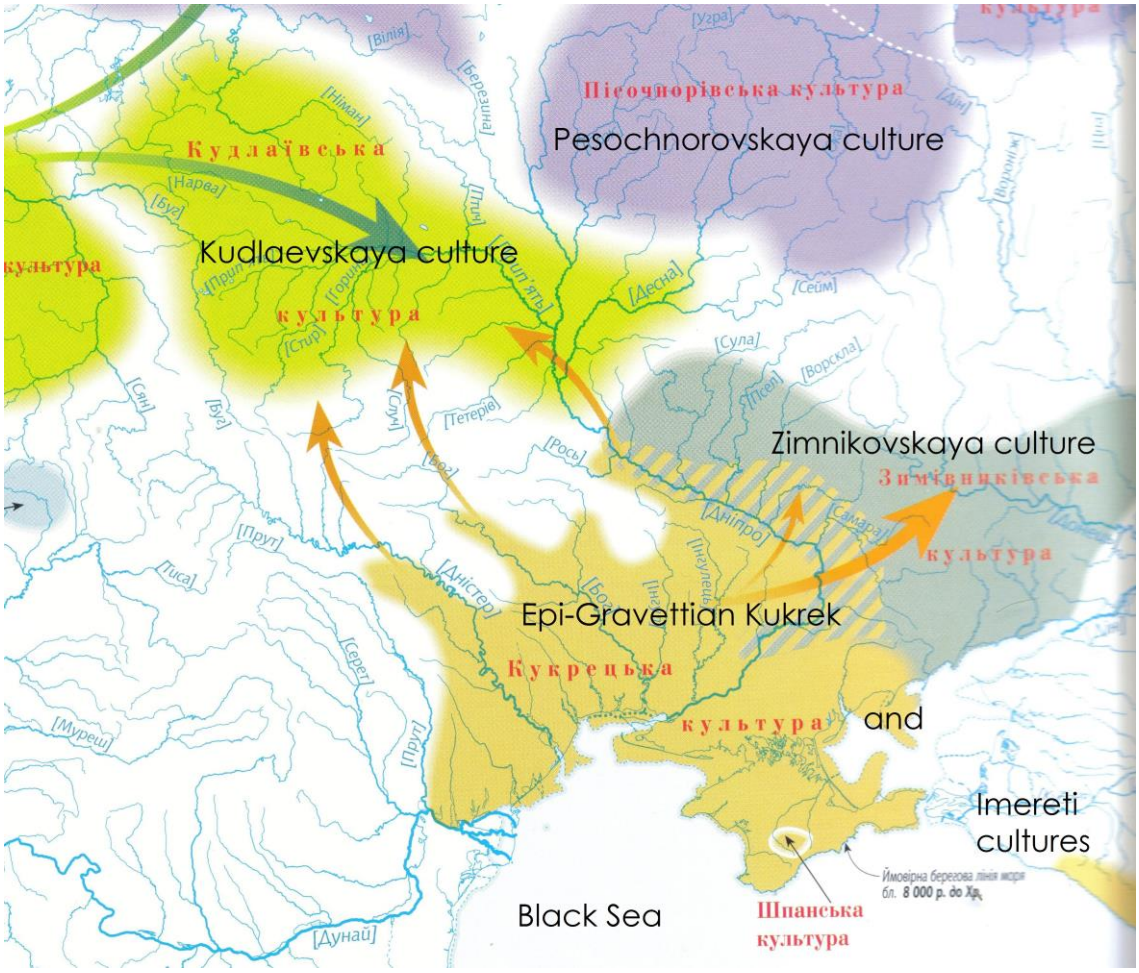

Figure 1. Black Sea area in the end of IX – first half of VII milleina BCE [29, p. 14].

Pre-Neolithic Bug-Dniester culture developed on the basis of the Kukrek one. As the above mentioned Satsurblia and Kotias burial grounds, genetics of which contains the male haplogroup J refer to the habitat of Imereti culture, we can make the conclusion that one of the subclades of the haplogroup J could have been spread across the North Black Sea Region in the pre-Neolithic period (common epi-Gravettian tradition). The mentioned migration by D.L. Gaskevich is supported by genetic data, if we consider that its representatives were the bearers of subclade J2b Y-haplogroup J. Nowadays subclade J2b is widely spread within the zone of ancient migrations of cardial tribes (Fig. 2).

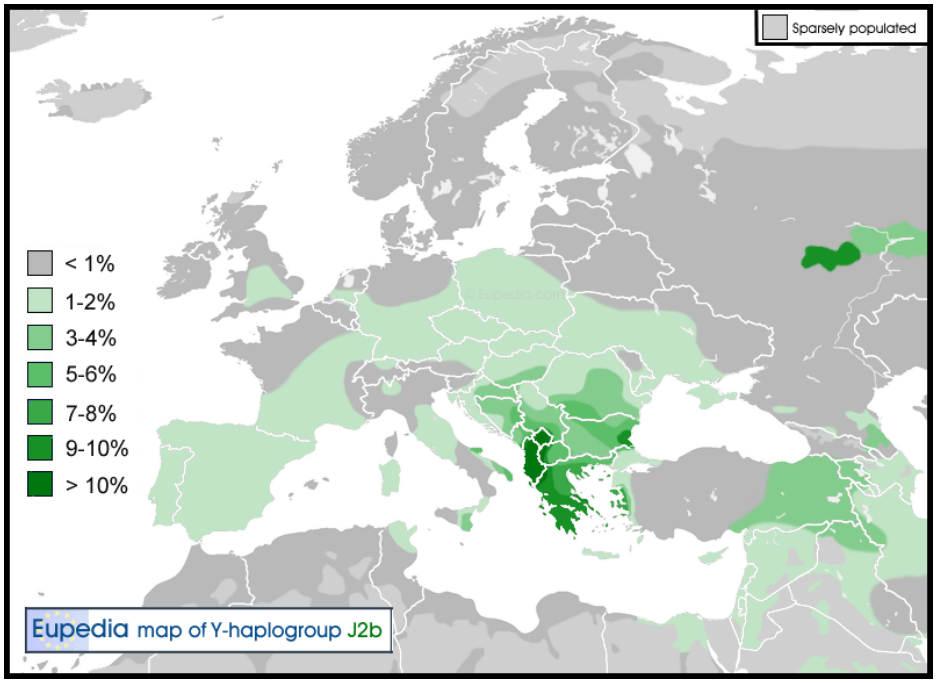

Figure 2. Distribution of haplogroup J2b (M102) in Europe, the Middle East & North Africa. http://www.eupedia.com/europe/Haplogroup_J2_Y-DNA.shtml

Those cardial tribes that could have given rise to the comb ceramics culture, may have been centered around Black Sea and Adriatic shores, and they could contain Y-DNA subclade which could be different with those of Georgia but related to them. The regions of the highest concentration of the haplogroup J2b bearers, namely Albania, the South-East of Bulgaria, Greece and some coastal regions of Italy (from 10 to 26 % of population) up to the Black Sea are represented on the above mentioned map.

This view is strongly supported by the spread of J2b in populations, which can be considered theoretically connected with the cultures of comb ware and were least of all Indo-Europeanized among all the East of Europe (nowadays they speak Uralic languages with some pre-Uralic substrate [30]). So, the presence of J2b in these populations may not reflect the Indo-European migrations, but earlier waves of Neolithic spread.

Firstly, the spot of the noticeable spread of J2b (10-15% of population) is Mordovia (central Russia) (particularly, Moksha environment [31]). V.V. Stavitsky in his thesis work on the theme «Neolithic, Eneolithic and the Early Bronze Age of Sura-Oka Interfluve and the Upper Prikhoper'e: Dynamics of North and South Cultures' Interrelations in the Forest-steppe Zone», *shows that the local population continued the development of the Upper-Volga comb-stroked traditions at the late stage of this culture existence without leaving the region. «The ornamentation of the ware of Ozimenki 2 site widely uses the broad-toothed prints of the long stamp with the rare rows of deep patches on top, having the complete analogs in the late Upper-Volga ware»* [32]. V.A. Yurchenkov in his book, which is the review of academic research, says about the prevailing comb nature of *Moksha river area* ware: «There are 20 memorials with the so-called comb-stroke ware in the Moksha basin. The prints of comb stamp prevailed in the ware decoration; the share of stroke ornament is low» [33, p. 113]. Thus, the penetration of J2b into Mordovia territory can be explained by the migration from the south, and the Mordovian J2b peak can be explained by the fact that the population of comb-stroke ware had not left the territory.

Secondly, Saami J2b phenomenon. The Saami have the reputation of the relict, some kind «reserve» of the ancient genes of Europe (and the bearers of the considerable pre-Uralic language substrate), that is why it is not surprising that one of the first Neolithic migrations to the European continent could be preserved in the genes of this isolated northern people, unaffected by «Indo-Europeanization» [34]. The population of the Saami within Kola Peninsula contains about 14% of haplogroup J2b [35]. As the Comb Ware cultures in pure form were displaced to the north of the

Eastern Europe in early Bronze Age, it is quite possible that their creators could have played an important role in the Saami formation.

New findings of 2016 year in Germany

Thus, we can suppose that haplogroup R1a1 could be found in the wide range of comb-stroke cultures, especially comb ware cultures often accompanied by J2b (at the north-west area – in Karelia and Mordovia exactly comb cultures exist in the considered period) in the Neolithic horizons. Besides, it is also possible that the epicenter of the spread and divergence of modern most widespread subclades R1a1 (M198+, M417+) was located to the west of Serteya, which is indicated by the relations of Serteya culture with the funnel beaker (developed on the basis of Ertebelle) and Narva cultures (Fig. 3).

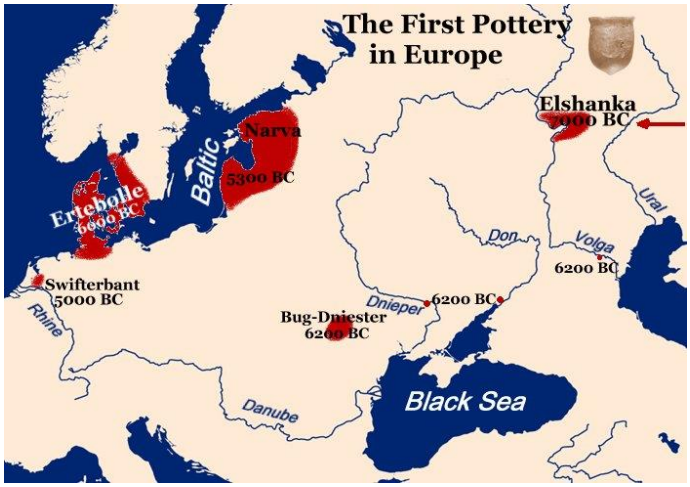

Figure 3. Main Ceramic Neolithic Cultures of Europe

But the issue of R1a1 bearers' Upper Paleolithic origin is debatable. Firstly, the variant of the Black Sea Region origin is still possible, as this haplogroup can be present in Bug-Dniester culture and further southward. This fact is supported by the detection of basal haplogroup in the population of the Middle East Region: «*... more basal (R1a-M420*) Y-chromosomes have been detected in Iran and eastern Turkey. Overall, our detection of haplogroup R1a1 in a northwest Russian hunter-gatherer establishes the early presence of this lineage in eastern Europe, and is consistent with a later migration from eastern Europe into central Europe which contributed such haplogroups to the Corded Ware population*» [5].

In [36] we discussed two possibilities. The first is that Y-DNA R1a1 could be ultimately a Zarzian marker which denotes the representatives of mesolithic cultures who came to Karelia from the South-East from the Caspian seashores (possibly via the Black sea region). The second is that R1a1 could come from the East or Central Asia in paleolithic time. The newest archaeological findings allow us to support the Zarzian point of view.

In the Northern Germany, where "*Mesolithic in different forms continued along with the existing agrarian societies of the Central Europe*" [37, p. 151], very interesting burial ground were found in recent years, which can be compared both with the Southern Scandinavia and Yuzniy Oleiny Ostrov burial (standing burials) (ibid). There is the theory that such unusual type of burial is the result of oriental influence (ibid), and this influence needs feasible explanation. Micro-blade techniques, which appeared in the Atlantic period and some forms of ware of the early V millennium BC also point at the oriental influence in the region (ibid). The priority of the oriental center of the mentioned influences in comparison with the west regions (Northern Germany) is determined by dating of the Yuzniy Oleiny Ostrov burial grounds (most of the burial grounds date back to the middle VI millennium BC [38, p. 307-464]).

Analogies of North German post-Mesolithic cultures and Yuzniy Oleiny Ostrov are found in such technology, as ware organic additives (fluff chamotte), which is rare for the early European Neolithic. Such types of ware are found within Karelia area in the beginning of V millennium BC

[39, p. 249] in Sperrings [40, p. 87], [41, p. 241-251]. As we mentioned above, Yuzniy Oleni Ostrov itself can be a ceramic culture. The Middle Volga is the earlier source of the 'fluff' technique. From places, where Elshanskaya archeological culture existed 6500 BC, this techniques spread from up the Volga to the Baltic Sea and further to the west to the location of the Ertebølle culture: «*Pottery was manufactured from native clays tempered with sand, crushed stone and organic material. The EBK [Ertebølle culture] pot was made by coil technique, being fired on the open bed of hot coals. It was not like the neighbouring Neolithic Linearbandkeramik and appears related instead to a pottery type that first appears in Europe in the Samara region of Russia c. 7000 cal BC, and spread up the Volga to the Eastern Baltic and then westward along the shore» (Fredrik Hallgren, The Introduction of Ceramic Technology Around the Baltic Sea in the 6th millennium, in Helena Knutsson, (ed.), Coast to Coast – Arrival, Coast to Coast book 10 (2004), pp. 123–142; Detlef Gronenborn, Beyond the models: Neolithisation in Central Europe, Proceedings of the British Academy, vol. 144 (2007), p.87; Jutta Paulina de Roever, The Pottery of Hunter-Gatherers in Transition to Agriculture, Illustrated by the Swifterbant Culture, the Netherlands in Dragos Gheorghiu (ed.), Early Farmers, Late Foragers, and Ceramic Traditions: On the Beginning of Pottery in the Near East and Europe (2009), pp. 150–166*» [42, pp. 123–142]; [43, p.87]; [44, pp. 150–166]. The origin of the Elshanskaya archeological culture is connected with large migration flow of the so-called 'Zarzian' cultures of the Middle East [45, p. 154] and possibly the spread of Dene-caucasian linguistic family [46]. Y-DNA haplogroup J [6], which is mainly spread within the Middle East also points at the southern nature of the population, as well as presence of mt DNA haplogroups J and H [2].

As we mentioned in our earlier work [47] the presence of eastern mtDNA C (which was attested in Yuzniy Oleniy Ostrov) is also attestable in southern regions (Dnieper-Donetsk culture). The Elshanskaya culture male sample was genotyped and Y-DNA haplogroup was R1b1-L278 [5]. In latter Khvalynsk culture we see both R1b1-M415 and R1a1-M459 [6] (and possibly M198-[15]), so the same picture could be possibly extrapolated to Elshanskaya (presence of R1a1-M459, M198-). The appearance of Karelia-type burials in the Ertebølle period and the spread of Elshanskaya-type ceramics with organic component allows to propose a hypothesis that the bearers of the new influences were the Zarzian migrants who moved west via North-Eastern Europe.

Another argument for Zarzian or related influence is that two other waves of Yuzhniy Oleni Ostrov populating are profoundly connected with Northern Europe and were already present near the Northern Germany region to the time of new burial style arrival.

In our previous work [46] we showed that besides Zarzian, the culture of Yuzhniy Oleni Ostrov burial can have other sources: Veret'e, Askoula-Suomusyarvi, Swiderian-Butovo cultures. The bearers of mitochondrial DNA U4 and U5a in the burial grounds point at the migrants from the regions of Europe. The emergence of U4 within Lake Onega has analogs in the Kunda culture in Lithuania (aged 6350 BC) [48], although Gotland island can also be the intermediate point (as mesolithic U4 were found there). MtDNA bearers of U4 in Germany also found in Bad Dürrenberg (aged 6850 BC) (ibid), in Sweden – Stora Förvar cave, Stora Karlsö Island [49]. Generally, subclade U4 was not still found in Paleolithic Western Europe [7], but exists in Central Europe eastward, for example, in Veret'e culture [3] (Popovo burial ground). Thus, its starting dispersal point could be connected with East-European final Paleolithic, even possibly Swiderian culture or its substrate, as Veret'e is similar to post-Swiderian cultures – Butovskaya and Kunda ones.

«*Veret'e culture (Oshibkina, 1983, 1989, 1997; Oshibkina, 1989) is spread within the East Prionezhe, mainly in the basin of the Lacha River. Three settlements of the Boreal time are well-studied, Veret'e 1 is concerned with the first and the Low Veret'e – with the second part of the Boreal. The lower layer of Sukhoe settlement and the poor Pogostishche site don't have natural-science dates. Besides, there is Peschanitsa burial ground, dated by the radiocarbon method 9890+-120 (GIN-4858) and Popovo burial ground. Burial grounds 9, 3 and 1 of the latter one date back 9730+-110 (GIN-4856), 9520+-130 (GIN-4442) and 9430+-150 (GIN-4447), in other words they date to the first half-middle Preborial and the burial ground in Peschanitsa – to its beginning. Burial grounds 6 and 8 date back 7510+-150 (GIN-3887) and 7150+-160 (GIN-4857) accordingly, in other words the Atlantic time. According to C.V. Oshibkina, the mentioned burial grounds belong to Veret'e culture. If it is true and the dates are correct, the excavated settlements characterize only the middle stage of this culture existence, which complicates the issue of its origin and historic fates. The similarity of the stone and bone goods and the synchronous*

memorials of Kunda and Butovo cultures enables to consider the similar development of Veret'e culture in Preborial, but this issue is still open, as well as the problem of its further development. Goods of Andozer M distinguish from the bone goods of Veret'e 1, which makes it impossible to refer the sites of this type to Veret'e culture. This site has more similarities with the late Mesolithic of the Sheksna basin of the type Ust-Anogi 1 (Kosorukova, 1997). At the same time, some similar features of goods enable to agree with the opinion of S.V. Oshibkina that the bearers of Veret'e culture were the ancestors of the population, which left the burial ground of Yuzhniy Oleni Ostrov. To solve the mentioned problems the search and excavation of new memorials is necessary. Meanwhile, we can note that the Boreal settlements of Veret'e culture, having a great number of different goods of bone and horn are one of the most impressing memorials of Mesolithic of the forest zone in the East Europe» [50].

U5 is the mitochondrial group, typical to the Upper-Paleolithic cultures like Gravettian one [51] and the dispersal routes of U5a bearers across Europe are concerned with the Central Europe (U5b prevailed in the west of Europe in Paleolithic-Mesolithic), rather than with the Western one. U5a seems to mark some migration (unknown to the archeologists) of the final Palelithic or early Mesolithic from the Danube (possibly where Dolni-Vestonice site was located, although this location dates back to the XXV millennium BC) (ibid) to Central Germany, where the route of the U5a bearers divides into two subclades U5a1 and U5a2.

Haplogroup U5a1 is the most probable for the U5a bearer in Yuzhniy Oleni Ostrov. The bearers of U5a spread far beyond Scandinavia and Baltics. Bearers of this haplogroup can be found in Chekalino culture – about 7800 BC [48], Lebyazhinka IV [5] culture of the VI millennium BC (ibid) and Lokomotiv burial ground of the Kitoy culture at the Angara (6100-4900 BC) [52]. The latter enables to affirm that the Kitoy culture, which some researchers consider as the ancestral for the Altai-language nation or at least for the part of it [53], may be connected with some Mesolithic migrations from the more westward regions. The analysis of the burial grounds of Bolshoi Oleniy Island [3], Lebyazhinka IV and the later cultures shows the existence of subclade U5a1 [5]. The work [3] shows similarity of populations of Yuzhniy and Bolshoi Oleniy Ostrov. This haplogroup was also found in Mesolithic Sweden (Motala burial). The latter two arguments make the existence of U5a1 in Yuzhniy Oleniy Ostrov the most possible.

Swedish burial ground Motala contains burials with a bunch of mitochondrial DNA: U2e, U5a1, U5a2, U5a2d [54, 9]. The fixed subclade U5a2 in Les Closeaux (Rueil-Malmaison) location, dated back to 8870 BC [7] and German subclade U5a2c3 in Blätterhöhle, dated back to 8638 BC [55] could be considered as the similar for Motala subclades. It is possible to consider that Motala subclades were brought from the Western or Central Europe in the period, synchronous to Yuzhniy Oleni Ostrov burial and the center of it dispersal was continental Western-Central Europe.

The possible candidate, which could have brought U5a to Yuzhniy Oleniy Ostrov is Askola-Suomusyarvi culture, possibly originating from final Paleolithic Ahrensburg culture (influenced by Hamburg final Paleolithic culture) [56, p. 185]. Though this culture differs from Motala, its population could more possibly have mitochondrial DNA U5a1, as well as the other cultures originating from Ahrensburg culture.

The possible tracks of mtDNA lineages to Karelia (Yuzhniy Oleni Ostrov), as they are seen according to contemporary data, are outlined on Figure 4.

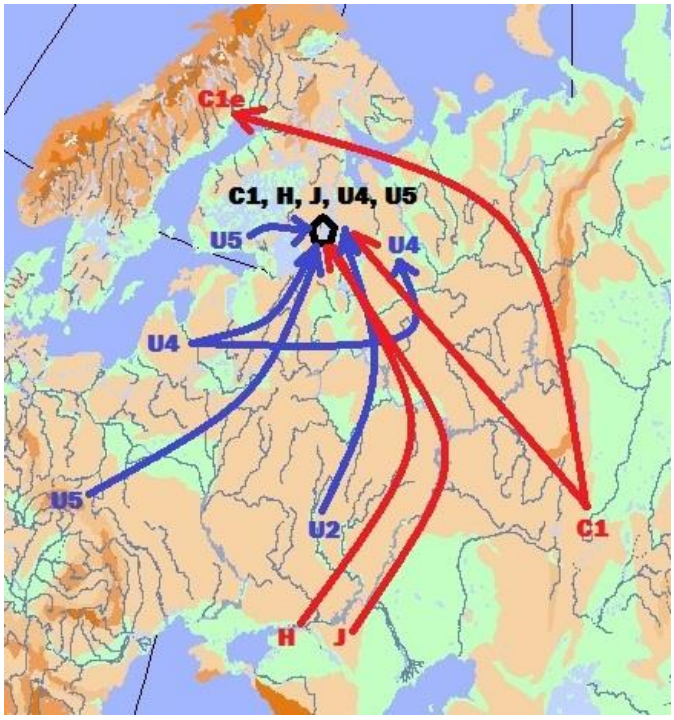

Figure 4. Possible ways of Mesolithic-Neolithic populating of Karelia (the territory of pre-Corded Ware R1a1* Y-DNA haplogroup presence)

4. Conclusion

We can conclude that the presence of haplogroup R1a1 is strongly probable in the cultures of Comb Stamp Ware (with J2b bearers as well) in the Neolithic context. But though the origin and the trace of R1a1 migration is still unclarified and more data are needed, the new archeological findings gives arguments for South-Eastern migration via the Baltic sea region to the Northern Europe which can be possibly accompanied with the Comb-Stroked Ware pottery with organic components.

References

1. Semenov A.S., Bulat V.V. Possible North-Eastern Connections of the R1a1-populations of Corded Ware Culture According to the Archaeologic and Paleogenetic Data // Russian Journal of Biological Research. 2015. Vol. 5, Is. 3, pp. 173-194.
2. Der Sarkissian C. et al. Mitochondrial Genome Sequencing in Mesolithic North East Europe Unearths a New Sub-Clade within the Broadly Distributed Human Haplogroup C1. February 4, 2014, DOI: 10.1371/journal.pone.0087612.
3. Der Sarkissian C. et al. Ancient DNA Reveals Prehistoric Gene-Flow From Siberia in the Complex Human Population History of North East Europe. http://repository.upenn.edu/cgi /viewcontent.cgi?article=1038&context=anthro_papers
4. Balanovsky O.P. Variability of the Gene Pool in Space and Time: synthesis of data, concerning gene geography of Mitochondrial DNA and Y-chromosome. Thesis for the Grant of the Degree of Doctor of Biology. M., 2012.
5. Haak W. et al. Massive migration from the steppe is a source for Indo-European languages in Europe. doi: http://dx.doi.org/10.1101/013433.
6. Mathieson I et al. Eight thousand years of natural selection in Europe. doi:http://dx.doi.org/10.1101/016477
7. Posth, C. et al. (2016), Pleistocene mitochondrial genomes suggest a single major dispersal of non-Africans and a Late Glacial population turnover in Europe. DOI: http://dx.doi.org/10.1016/j.cub.2016.01.03
8. Chekunova E.M., Yartseva N.V., Chekunov M.K., Mazurkevich A.N. The First Results of the Genotyping of the Aboriginals and Human Bone Remains of the Archeological Memorials of the Upper Podvin'e. // Archeology of the lake settlements of IV—II Thousands BC: The chronology of

cultures and natural environment and climatic rhythms. Proceedings of the International Conference, Devoted to the 50-year Research of the Pile Settlements on the North-West of Russia. St. Petersburg, 13–15 November, 2014.

9. Eppie R. Jones et al. Upper Palaeolithic genomes reveal deep roots of modern Eurasians. Nature Communications. DOI: 10.1038/ncomms9912 | www.nature.com /naturecommunications.

10. Rui Bi at all. Mitochondrial DNA haplogroup B5 confers genetic susceptibility to Alzheimer's disease in Han Chinese. Neurobiology of Aging 36 (2015), http://www.mitotool. org/lab/pdf/Binder1.pdf

11. Romanchuk A.A., Semenov A.S. R and Q haplogroups of Y-chromosome and Proto-North Caucasian Substratum of Proto-Indo-Europeans // Russian Journal of Biological Research, 2014, Vol. (1), № 1, pp. 46-68

12. Underhill P.A. The phylogenetic and geographic structure of Y-chromosome haplogroup R1a./ European Journal of Human Genetics (2014), 1–8.

13. Firasat, Sadaf; Khaliq, Shagufta; Mohyuddin, Aisha; Papaioannou, Myrto; Tyler-Smith, Chris; Underhill, Peter A; Ayub, Qasim (2006). "Y-chromosomal evidence for a limited Greek contribution to the Pathan population of Pakistan". European Journal of Human Genetics 15 (1): 121–6.

14. Nasidze et al., (2004) Mitochondrial DNA and Y-Chromosome Variation in the Caucasus. Annals of Human Genetics (2004) 68, 205–221. https://www.familytreedna. com/pdf/caucasus.pdf

15. https://genetiker.wordpress.com/2015/11/24/more-y-haplogroups-for-prehistoric-eurasian-genomes/

16. Yakimov V.P. Anthropologic Data from Neolithic Burial Ground at the Yuzhny Oleniy Island // Proceedings of the Museum of Anthropology and Ethnography. M.–L. V.XIX. 1960.

17. Filatova V.F. Oleniy Island Burial Ground in the System of Mesolithic Settlements of Karelia.// Kizhi Journal No.7. Editorial board: I.V.Melnikov (editor-in-chief), R.B. Kalashnikova, K.E. German. Reserve museum «Kizhi». Petrozavodsk. 2002.

18. Tretyakov P.N. Finno-Ugrians, Balts and Slavs at the Dnieper and Volga. M., 1966.

19. Zubova A.V. The Population of Yamna cultural-historical community in considering the odontological. // Vestnik arkheologii, antropologii I etnografii. 2010. № 2 (13).

20. Mazurkevich A.O. On Ancient Cultures in Pskov area. http://gubernia. pskovregion.org/number_27/7.php

21. Miklyaev A.M. Stone and Iron Ages in the Interfluve of the Western Dvina and Lovat. // St. Petersburg's Archeological Journal. Issue 9. St. Petersburg, 1994. (http://cheloveknauka. com/v/498796/a#?page=1)

22. Tartarica. Atlas. Kazan, 2005.

23. Chernyavsky M.M. Usvyaty culture // The Archeology of Belarus: Encyclopedia. Vol. 2, Minsk, 2011.

24. Neolithic of the Northern Eurasia. M., 1996.

25. Malov H.M. The Archeology of Volga Region. Saratov, 2012.

26. Vasilev I.B. Khvalynsk Eneolithic Culture of Volga-Ural Steppe and Forest-Steppe. // The Issues of Volga Region Archeology. Issue 1. Samara, 1999.

27. Gaskevich D.L. The North-Pontic Impresso: the Origin of Neolithic Patching-comb at the South of West Europe. Stratumplus. No. 2. 2010.

28. Loza Yu.I. The Historical Atlas of Ukraine. The Recent and the Ancient One. Rus. (Kiev Rus, Galitsko-Volinskaya State). V 1. Kiev, 2015.

29. Loza Yu.I. The Historical Atlas of Ukraine. V 2. Kiev, 2015.

30. http://starling.rinet.ru/confer/Zhivlov-2015.pdf
http://starling.rinet.ru/confer/Zhivlov-2015.pdf

31. Trofimova Natalya Vadimovna. Variability of Mitochondrial DNA and Y-chromosome in Volga-Ural Region Population: Thesis work of PhD of Biology: 03.02.07 / Trofimova Natalya Vadimovna; [The place of defense: The Institute of Biochemistry and Genetics of Ufa Scientific Center of the Russian Academy of Sciences http://ibg.anrb.ru/dissov.html].

32. Stavitsky V.B. Neolithic, Eneolithic and the Early Bronze Age of the Sura-Oka Interfluve and the Upper Prikhoper'e: Dynamics of Interaction of the Cultures of the North and the South in the Forest-steppe Zone. Penza, 2005.

33. Yurchenkov V.A. Mordovian Region. Popular Archeology: Remote Ancestors (review publication). Saransk, 2013.

34. Kristiina Tambets et all. The Western and Eastern Roots of the Saami – the Story of Genetic "Outliers" Told by Mitochondrial DNA and Y Chromosomes. Am. J. Hum. Genet. 74: 661–682, 2004.

35. Raitio M, Lindroos K, Laukkanen M, Pastinen T, Sistonen P, Sajantila A, Syvanen A (2001) Y-chromosomal SNPs in Finno-Ugric-speaking populations analyzed by minisequencing on microarrays. Genome Res 11:471–482.

36. A.S. Semenov, V. V. Bulat. On Localization of Ancient Bearers of Y-DNA R1a Haplotype in Eastern Europe Neolithic Cultures. Russian Journal of Biological Research, 2015, Vol. (6), Is. 4, pp. 227-240. DOI: 10.13187/ejbr.2015.6.227

37. Thomas Terbergeret all. Standing upright to all eternity – The Mesolithic burial site at GrosFredenwalde, Brandenburg (NE Germany). Aufrecht in die Ewigkeit – Der mesolithische Bestattungsplatz von GrosFredenwalde, Brandenburg (Nordostdeutschland). Quartar 6 doi: 10.7485/QU62_6 2 (2015): 133-153.

38. Karelia: the encyclopedia. Volume 2. Petrozavodsk, 2009.

39. Khoroshun T.A. Kulkova M.A. The Peculiarities of the Making of Clay Pottery in the Late Neolithic on the Territory of Southern Karelia. // Archeology of the lake settlements of IV—II Thousands BC: The chronology of cultures and natural environment and climatic rhythms. Proceedings of the International Conference, Devoted to the 50-year Research of the Pile Settlements on the North-West of Russia. St. Petersburg, 13–15 November, 2014.

40. German K.E. The Archaeological Artifacts with Sperrings Ceramics in the Onega Lake Bassin:the abstract on the Ph.D. thesis. Saint-Petersburg: 2001.

41. German K.E. The History of the Studing of Sperrings Culture in Finland. // The Vestnik of Kizhi, № 6, 2001.

42. Fredrik Hallgren, The Introduction of Ceramic Technology Around the Baltic Sea in the 6th millennium, in Helena Knutsson, (ed.), Coast to Coast – Arrival, Coast to Coast book 10 (2004).

43. DetlefGronenborn, Beyond the models: Neolithisation in Central Europe, Proceedings of the British Academy, vol. 144 (2007).

44. Jutta Paulina de Roever, The Pottery of Hunter-Gatherers in Transition to Agriculture, Illustrated by the Swifterbant Culture, the Netherlands in DragosGheorghiu (ed.), Early Farmers, Late Foragers, and Ceramic Traditions: On the Beginning of Pottery in the Near East and Europe (2009).

45. Mesolithic of the USSR. M., 1989.

46. A.S. Semenov, V. V. Bulat. Some Conjectures on Y-DNA Haplotype R1a1 Migrations Based on new North Eurasian Paleogenetic Data. Russian Journal of Biological Research, 2015, Vol. (4), Is. 2, pp. 85-98.DOI:10.13187/ejbr.2015.4.85

47. Semenov A.S., Bulat V.V. Possible North-Eastern Connections of the R1a1-populations of Corded Ware Culture According to the Archaeologic and Paleogenetic Data. Russian Journal of Biological Research, 2015, Vol. (5), Is. 3, pp. 173-194. DOI: 10.13187/ejbr.2015.5.173

48. Bramanti, B. et al. (2009), Genetic Discontinuity Between Local Hunter-Gatherers and Central Europe's First Farmers. *Science* 02 Oct 2009: Vol. 326, Issue 5949, pp. 137-140. DOI: 10.1126/science.1176869.

49. Skoglund, P. (2013), Reconstructing the Human Past using Ancient and Modern Genomes, Dissertation Uppsala University. urn:nbn:se:uu:diva-206787 (http://urn.kb.se/resolve ?urn=urn:nbn:se:uu:diva-206787).

50. http://mizhilin.narod.ru/Bone_Industry_2001/Chapter_1.htm

51. Fu, Q. et al. A revised timescale for human evolution based on ancient mitochondrial genomes, Current Biology, 21 March 2013.

52. MooderK. et al. (2006), Population Affinities of Neolithic Siberians: A Snapshot From Prehistoric Lake Baikal, *American Journal of Physical Anthropology*, vol. 129, no. 3 (March 2006), pp. 323-481.

53. Turov M.G. More on the historical Urheimat and early ethnogenesis of Tungusians // Peoples and Cultures of Siberia. Communication as the Factor of Formation and Modernization: (papers). Irkutsk. C 147-180.
54. Lazaridis, I. et al. (2013), Ancient human genomes suggest three ancestral populations for Europeans. DOI: http://dx.doi.org/10.1038/nature13673.
55. Bollongino, R. et al. (2013), 2000 years of parallel societies in Stone Age Central Europe. *Science* 25 Oct 2013: Vol. 342, Issue 6157, pp. 479-481. DOI: http://dx.doi.org/10.1126/science.1245049.
56. Mongayt A.L. The Archaeology of the Western Europe. The Stone Age. M., 1973.

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Древние палео-ДНК Северо-Восточной Европы: к реконструкции миграций носителей R1a1 до начала энеолита

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Аннотация. В данной работе рассмотрены основные вопросы палеогенетики, археологии и антропологии, связанные с заселением Восточной Европы в мезо- и неолите. В данных, собранных различными лабораториями и коллективами, содержится определенное количество образцов различных мтДНК и Y-ДНК гаплогрупп рассматриваемого периода (типированные останки), что позволяет выстроить связи между носителями культур соседних регионов. Это достигается привлечением археологических данных, что позволяет отследить и миграционные потоки. В статье делается попытка построить картину некоторых миграций эпохи раннего неолита (связанных с перемещением носителей гаплогруппы R1a1). Делается вывод, что обнаружение новых древних останков с Y-гаплогруппой R1a1 возможно в слоях культур восточно-европейской гребенчатой керамики, которая в свою очередь может быть связана с неолитом Причероморья. Статья может быть использована для биомедицинских исследований, поскольку в настоящее время уже выявленны отдельные корреляции между гаплогруппами и наследственными болезнями.

Ключевые слова: Y-ДНК гаплогруппа, R1a1, J2b, митохондриальная гаплогруппа, U4, U5a1, палеогенетика.

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