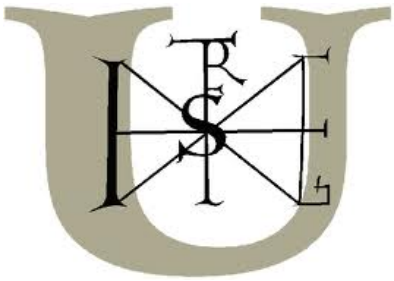

SZENT ISTVÁN UNIVERSITY

THESIS OF PHD DISSERTATION

CONSERVATION BIOLOGY RESEARCH ABOUT THE CASPIAN WHIPSNAKE POPULATIONS IN HUNGARY

(SQUAMATA: COLUBRIDAE: *DOLICHOPHIS CASPIUS*)

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1 THE ACTUALITY AND SIGNIFICANCE OF THE TOPIC

The Caspian whipsnake *(Dolichophis caspius*) had been one of the least known reptile species until the end of the 20th century. Even though the species got its Hungarian name based on ethological observations, we do not have unequivocal, reliable data about its biology or its way of living or even about its distribution in the Carpathian Basin. Our little knowledge cannot only be attributed to the hiding way of life of the Caspian whipsnake. The research of the herpetologists interested in this species was also rendered difficult by the fact that it is hard to access the habitats of this species or to keep it in a terrarium (DELY 1997). The first work to supply this need presents the herpetofauna of the highly protected Szársomlyó Nature Reserve and also gives a partial account of the biggest Hungarian population of the Caspian whipsnake (DUDÁS 2001). A conservation plan - partially based on this work and on our previous data-collection - has been designed for this species, too, coordinated by the Ministry of Environment and Water Management (BAKÓ, BELLAAGH 2004) which serves as a basis for carrying out the protection plan of the individual populations. For the reconstruction and efficient protection of the species' habitats it became indispensable to conduct a thorough survey about the habitat needs of this species.

The exact taxonomical position of the Central European - and at the same time the Hungarian - Caspian whipsnake populations has been the focus of many researches (NAGY *et al.* 2004) and many debates until now. Neither the systemic category of the species nor the relationship between the populations living in Hungary and in the territories south of Hungary, in the Balkan peninsula or in Anatolia have been defined.

The Hungarian Caspian whipsnake populations are the northest and at the same time the most western populations of the species, which must have reached the Carpatian Basin from the populations in the Balkan Peninsula and Anatolia following the dispersion after the last glacial period. After this, the Hungarian populations must have become isolated from one another due to the gradual fragmentation of their habitats. The previous natural habitats of the species in Hungary have gradually disappeared or become fragmented. They have been replaced by settlements and agro-ecosystems and other habitats, which provided different living conditions. For the protection of the remaining habitats it became more and more indispensable to summarize

those factors that might pose a threat on the habitats. This, together with the habitat needs of the species constitutes a highly important input for the management plans designed for the individual habitats.

The Caspian whipsnake, which has been regarded until now as a species with a particularly low environmental tolerance, has appeared in new, unregistered localities in the last few years (KORSÓS *et al.* 2002). These localities are the fragments of former, presumably bigger habitats; the latter ones can be regarded as fragments situated at the matrix of urbanised and agricultural areas. Supposing that the recent habitats are the remains of an earlier and bigger habitat it became indispensable to examine the potential habitat patches with similar living conditions not only in the Buda hills but also in the surroundings of the recently discovered population in Paks.

2. DEFINING PROBLEMS AND GOALS

Based on the above mentioned data and defects the aim of my work was to provide some new information about the Caspian whipsnake, one of the least known and most rare reptile species in Hungary, which can be useful both for natural scientists and environmentalists.

At the beginning of my research I defined the following goals:

- To unify all the findings about both the former and the current Caspian whipsnake populations from specialised literature and from fieldwork, thereby giving an up-to-date picture of the distribution of the species in Hungary,
- To identify those parameters in the natural habitats which might indicate the presence of the Caspian whipsnake on a given territory,
- To provide numerical data about the developmental stability, the unaffectedness and the quality of the living conditions of the biggest Hungarian Caspian whipsnake population, using fluctuating asymmetry analyses,
- To define those natural and anthropogenic influences that endanger the Hungarian Caspian whipsnake populations and to put forward some suggestions for the efficient protection of these habitats,
- To reveal - using the methods of molecular genetics - those genetic relationships among

the individual Caspian whipsnake populations in Hungary that can serve as a basis for reconstructing the colonisation-route of the species in the Carpathian Basin and its distribution history.

3. MATERIALS AND METHODS

Surveying the Hungarian habitats of the Caspian whipsnake

In order to give an up-to-date picture of the distribution of the species in Hungary I identified the habitats on the maps used in the first, second and third military surveys and then on the successive civil maps, using contemporary accounts of the species. I regularly examined those habitat-fragments which remained intact, based on the data I gathered from the maps, from specialised literature (DOBOLYI 2001), and from my previous fieldworks. I observed the habitats every two months from spring to autumn, when the Caspian whipsnakes were casting their skin, and between April and September. Apart from the two habitats which had been known long before (the Buda hills and Szársomlyó) a new habitat of the species has been discovered near Paks (KORSÓS *et al.* 2002). Taking the vegetation of the territory as a basis and using aerial photographs I have defined those fragments that are situated at the border between Mezőföld and the Danube. These can be regarded as further potential habitats of the species. My research of the eastern periphery of Mezőföld is grounded on these findings. The areas involved in the research were the following: the loess-areas between Dunaújváros and Kisapostag, the loess walls at the Danube-bank, north from Dunaföldvár, the loess walls between Bölcske and Madocsa, the vineyard between Paks and Dunakömlőd, Dunaszekcső and Várhegy.

Fluctuating asymmetry

During my research in the Villány hills I registered several morphological characters (the number of the periocular, supralabial, sublabial, loreal, nasal, dorsal, ventral and subcaudal scutums). From these I have used the numerical values of five characters; those of the periocular, supralabial, sublabial, loreal and nasal scutums on the left and right side of the body. I used the index-values calculated from the characters to determine the developmental stability of the Caspian whipsnake population in Villány. In order to describe the developmental stability of this

population – which also involves both the environmental stress-factors and those that originate from within - of the Caspian whipsnake population in Villány hills we needed a basis for comparison. As there is no other Caspian whipsnake population in Hungary which includes as many specimen as the one in Villány, I compared the indexes calculated from the data on the Caspian whipsnake with the indexes derived from the morphological data on dice snakes *(Natrix tessellata* LAURENTI, 1768*)*. This data has been gathered from a highly disturbed habitat (Mád) and an extensive, fairly intact habitat (Balaton) (HERCZEG *et al.* 2005). I have used the same morphological features of both the Dice snake and the Caspian whipsnake during the analysis. In the following analysis I defined the value of the FA11 and the FA12 asymmetry indexes based on more morphological features, and those of the FA1 and FA5, based on one morphological feature (PALMER 1994). The complex indexes made the combined comparison of distinct morphological characters possible. I also made a comparison of the three populations (Szársomlyó /Villány/, Mád and Balaton) based on the absolute morphological asymmetry – $|J-B|$ – among the individual specimen, without calculating the indexes. By this I wanted to assess the efficiency (i.e. the ability to indicate asymmetry) of the morphological features I used and to describe the influence they exert on one another.

Habitat preference

I gathered field data for the analysis of habitat preference in Szársomlyó. I started the survey on a territory of 51 quadrates. I indicated 36 points (S) on the area where the Caspian whipsnake is present and designated 15 random points (R) on the paper-based maps. I estimated the habitat-structure parameters of the examined territory in 2x2 and 5x5 meter quadrates. In the 2x2 meter quadrates I examined the herbaceous plant coverage, the woody plant coverage, the height of the grass, the proportion of free rock surface and free soil surface and the steepness of the area. I examined the edge length of the vegetation, using 5x5 meter quadrates. The centre of these quadrates corresponded to the centre of the 2x2 meter quadrates. I analysed the role that the structure of the landscape has in the habitat choice of the species on a bigger scale, too. For the big-scale analysis of the landscape structure I drew circles around the S and R points with a radius of 50 and 100 meter. I identified the woody plant patches within these circles, using the ArcWiew (ESRI 2000) program. Based on the data I calculated the proportion of herbaceous and woody plant coverage and the edge length. I also examined the correlation between the habitat

choice of the Caspian whipsnake and the steepness of the area. As a basis for the analysis I used the previously designated points and the surrounding 25, 50 and 100 meter radius circles. I calculated the data describing the steepness of the area with the help of MapInfo program, using net slope database of a 1, 5 x 1, 5 meter resolution.

Identifying the threat-factors of the individual populations

I have placed the threat-factors of the individual Caspian whipsnake habitats in Hungary into two categories according to their origin: anthropogenic and natural. I indicated the influence that the individual factors exert on a given habitat with 0 or 1, according to their presence or absence. Having determined the degree of endangeredness of the individual habitats I weighted the data about them according to the degree of their legal protectedness. As the size of the individual habitats is another factor that influences the survival chances of the populations, I have placed the habitats into three categories, based on their territorial extension and I indicated the corresponding numbers in the table. I assigned a numerical value to the endangering factors based on their occurance in the individual habitats. The maximum theoretical value of this corresponds to the number of the Caspian whipsnake habitats involved in the analysis. This means that the more habitats are affected negatively by a given factor, the higher the assigned value is. The total numbers of the endangering factors that are present in a given habitat correspond to the numbers indicating the endangeredness of the habitats. In this way the legal protectedness of the area has also been taken into account.

Genetic analyses

For the genetic analyses I used the blood samples I had taken during the fieldwork, cast-off scales, blood samples taken from dead specimen, and the blood samples of the specimen from the public collection and from the isolated DNA samples from the gene-bank. I examined the specimen from the public collections of the National Museum of Natural Science and from the herpetological collection of the Museum of Natural Science in Vienna and in Belgrade. When choosing the samples - before isolating the DNA – the main criteria were their territorial representativeness and their freshness. I cleaned the DNA using the standard phenol-chlorophorm method (SAMBROOK *et al.* 1989, BRICKER *et al.* 1996). The sequentation of the isolated extranuclear citocrome b proteoltic gene taken from the tissue sample of the Caspian whipsnakes

was conducted at the molecular genetics laboratory of the Royal Belgian Institute of Natural Sciences, within the frameworks of a project by the National Innovation Office (3B023-04), project entitled "The zoological values, zones and genesis of the Carpathian Basin". The sequences provided by the institution were evaluated in Hungary with the help of the programmes MEGA4 (TAMURA *et al.* 2007) and PAUP*4.0b10 (SWOFFORD 2002).

The sequences were compiled of 46 samples and they were 1117 base pair long. First I conducted a simple Neighbour Joining analysis in order to determine which samples belong to the same haplotype. I chose one of the samples of the same haplotype and I always used the sequences of the same specimen during the further analysis. I applied the following methods: Maximum Parsimony, Neighbour Joining, Maximum Likelihood, Bayes-phylogenesis, TCS analysis.

4. RESULTS

Mapping the habitats of the Caspian whipsnake in Hungary

The new Caspian whipsnake habitats discovered during my examinations are the following: Szállás hill (Buda hills), Dunaszekcső, Dunaújváros, Dunaföldvár (BELLAAGH *et al.* 2008).

The result of the examination of the individual habitats with regard to the occurrence of the species are summarised in the table below. The detailed descriptions of the individual habitats are included in the doctoral thesis.

Habitat	Current	Extinct	New discovery
Újlaki hill		**X**	
Mátyás hill		**X**	
Sas hill	**X**		
Csíki hills	**X**		
Szállás hill	**X**		**X**
Törökugrató		**X**	
Dunaújváros	**X**		**X**
Dunaföldvár	**X**		**X**
Paks - Dunakömlőd	**X**		
Dunaszekcső	**X**		**X**
Szársomlyó	**X**		

Based on the experiences of the fieldworks we can localise further potential habitats where the loess-areas of Mezőföld and the Danube are vertically structured enough. Other areas to be included in the examination can be found on the eastern edge of Mezőföld, on the right bank of the Danube. These include the loess areas around Kulcs and Rácalmás and between Érd and Százhalombatta, which show significant similarities with the habitats in Dunaföldvár and Paks. Providing that are able to establish the presence of the species in these territories, the distribution map of the Caspian whipsnake can be broadened by two new UTM squares.

Fluctuating asymmetry (FA)

On comparing the FA values calculated from the data on the sublabial scutum of the individual populations we found that the Dice Snake populations of Mád and those of the Lake Balaton significantly differ in terms of their FA1 and FA5 index values. The FA value calculated from both indexes was higher in the Mád population than in the Balaton population. A significant difference was detected between the FA1 and FA5 indexes of the Mád and the Villány populations. The values of the FA indexes were significantly lower in the Caspian whipsnake population of Villány than the corresponding values in the Dice Snake population of Mád. Between the Dice Snake population of Balaton and the Caspian whipsnake population of Villány no significant differences were detected in the FA1 and FA5 index values. The results we got after comparing the FA11 and FA12 values of the complex indexes were similar to those presented above. The simple index values derived from the data about the periocular scutum did not indicate any significant differences among the populations. The developmental stability of the two examined features (i.e. the sublabial and periocular scutum) does not show significant differences. I established that the differences among the three populations can be detected even if we only examine the sublabial scutum because the data of the sublabial scutums predict the degree of asymmetry in the individual populations. The values of the two morphological characters do not show correlation.

Habitat preference

With the help of the methods used during the data-evaluation we were able to identify the main characteristics of a habitat, which may indicate the occurrence of the Caspian whipsnake on a

given territory. In the case of the characteristics measured in 2x2 quadrates we examined the height of the grass, the herbaceous and woody plant coverage and no significant differences were detected between random points and those that are demonstrably used by the Caspian whipsnake. The values of free rock surface and free soil surface showed significant differences between the two groups. The differences in the values of steepness were significant between the S (snake) and R (random) points. The territories used by the snakes were steeper than the random ones. In terms of edge length we found significant differences between the randomly designated points and those that are demonstrably used by the Caspian whipsnake. The snake preferred the territories with a higher value of edge length. Among the territories (with a radius of 25, 50 and 100 m) surrounding the S and R points a significant difference was found in the edge-lengths. In the case of the areas demonstrably used by the Caspian snake the edge-length was bigger than in the case of random points. After analysing the proportions of grassland on a similar scale, I found a significant difference only in the case of the territories with a 100 meter radius, where a higher value was assigned to the random points. There is an inverse relationship between the proportion of the grassland and the occurrence of the snake, while a positive correlation was detected between the presence of woody plants and the presence of the snake. In terms of steepness a significant difference was detected between the random points and those used by the snakes in the case of both the 25 and the 50 meter radius territories.

Identifying the endangering factors of the individual habitats

The maximum theoretical value of the endangeredness of the examined Caspian whipsnake habitats was 24 points. The highest point (14) has been reached by the small-sized habitats outside the protected areas. Based on the results indicating the degree of endangeredness I placed the recent Caspian whipsnake populations into three categories. Areas with 1-4 points fall into the "not directly endangered" category. This group only contains territories which are already protected by law (Szársomlyó, Sas hill). Those areas which scored between 5-10 points belong to the category of endangered areas (Farkas hill, Szállás hill, Mátyás hill, Paks) but it would be still possible to save them by introducing security measures and implementing habitat-reconstruction works. Territories which exceeded 10 points on the scale can be regarded as highly endangered habitats Dunaföldvár, Paks, Dunaszekcső). These areas are currently unprotected and are threatened by both human and natural influences. Therefore, providing legal protection must be the first step towards the conservation of these territories. Apart from the size of the territory, the

most influential and thereby the highest scoring factors are the diffusion of invasive plants and human disturbance. These two factors are closely followed by the occupation of the land by agriculture. Other risk-factor include external mining and construction and in the case of the habitats in Dunaújváros and Dunaföldvár off-road motorcycling. The small size of these territories constitutes another potential source of threat, as smaller territories are less resistant to either human influences or natural processes.

Genetic analyses

From 49 tissue samples it was possible to extract the DNI of suitable quality and quantity that we needed for further analyses. The samples represented all the eastern European and Anatolian territories where the Caspian whipsnake occurs. After sequenting the cit-b gene in its full length (1117 bp) I was able to identify 9 different haplotypes using simple Neighbour Joining Methods. The haplotypes within the sub-groups are determined by 147 variable positions. During the analysis of Maximum Parsimony I have found 23 parsimonically informative characters. By analysing the relationships among the haplotypes we identified two main cladi. The difference between the two main cladi was significant in the case of all the three methods. However, the relationships within the individual cladi are not clear. One of the main cladi includes the populations located east of the Aegean Sea and the Sea of Marmara. The other cladus consists of the samples from the European habitats. The latter one includes the samples from territories extending from Southern Greece till the Sas hill. The populations deriving from the islands of the Aegean Sea belong to the H8 and the H9 haplotype and comprise a "transitional" population between the two cladi. The relationships within the individual cladi are not clear due to the lack of enough evidence. Only the samples of the H1 haplotypes from the island of Andros are significantly distinct from the rest of the "European" cladus. The analyses conducted by TSC 1.21 programme also resulted in two main cladi, which significantly differ from one another. The populations located near the Bosporus and on the eastern islands of the Aegean Sea are kins, while the European populations constitute a significantly different group. The group H5 can be regarded as the most ancient haplotype within Europe.

New scientific findings

- I have given an up-to-date picture of the occurrence of the Caspian whipsnake population in Hungary. I have mapped new habitats that have not been registered yet.

- Having examined all the known habitats of Caspian whipsnake in Hungary I identified those environmental factors that might endanger the individual habitats and indirectly the Caspian whipsnake populations, which live there and which are highly protected. These factors are the following: the shrinking of the habitats, open-pit mining, direct cultivation of the land, construction, illegal visits and waste-dumping, the transformation of the vegetation structure due to invasive plant species, succession and erosion.
- I placed the habitats into different categories, according to the degree of threat they are exposed to. I have made some suggestions which can serve as a basis for taking measures to ensure survival of these habitats.
- After carrying out the habitat preference analyses I have surveyed those parameters of the habitats which can serve as a basis for predicting those habitats of the Caspian whipsnake which have remained undiscovered until now. I have demonstarted that the Caspian whipsnake prefers steep habitats and territories with a strong vertical structure even when examined at a lower level. It also prefers habitats with an edge-like vegetation, therefore, during the reconstructional operations it would facilitate the survival of the species if these kind of habitats could be preserved or re-created. Another positive correlation can be established between the occurrence of the species and the free rock surface in the habitats. By having done an indirect habitat preference analysis I have provided detailed information about the habitat needs of the Caspian whipsnake, which might serve as a basis for environmental protection and habitat-reconstruction plans.
- By conducting a fluctuating asymmetry analysis I have provided information about the developmental stability of the biggest Hungarian Caspian whipsnake population. I have established that the value of the fluctuating asymmetry in the Caspian whipsnake population do not differ significantly from that of the Dice Snake population, living in an undisturbed habitat. Thus, the population in Szársomlyó boasts a relatively big developmental stability.
- Using the methods of molecular biology I have revealed the genetic relationships among the Caspian whipsnake populations in Hungary and I have theorized the potential recolonisation route the species might have used after the glacial period. Based on the results we can identify two big haplotype-circles on the diffusion territory of the Caspian whipsnake. A relationship can be established between the populations of the Carpathian

basin and the populations of the Balkan Peninsula, which belong to haplotype H5. Thus, the theory, according to which the species survived the glacial period in the refuge localized on the Balkan Peninsula, has been confirmed.

- I have established that the Caspian whipsnake population in Szársomlyó displays significant genetic differences compared to the other Hungarian populations (of Buda, Paks, Dunaújváros and Dunaföldvár). The Szársomlyó haplotype (H6) significantly differs from haplotype H5, the latter of which is widely diffused and includes habitats extending from the Sas hills till the Duna estuary and the Balkan. This difference from the H5 haplotype is more significant than the split of the east-balkanic (H4) and the Thracian (H7) haplotypes. The explanation of this latter phenomenon can be found in the increasingly declining genetic diversity which characterises the isolated, in genetic terms island populations (Szársomlyó H6).

5 CONCLUSIONS AND RESULTS

The distribution map of the Hungarian Caspian whipsnake populations

Based on the Caspian whipsnake populations discovered during the research period it can be understood that in Hungary this species has a higher occurrence rate than it was previously estimated (DELI 1997, BAKÓ-KORSÓS 1999). However, it should still be considered as a highly protected species due to its rare occurrence and the endangeredness of its habitats. Based on the fieldworks we can presume the existence of further habitats on those loess territories in Mezőföld and close to the Danube bank that are vertically structured so in the future the mapping of two further locations can be justified, that is the loess walls between Kulcs and Rácalmás and the territories between Érd and Százhalombatta. If the presence of the species can be established in these territories, the distribution map of the Caspian whipsnake can be broadened by two new UTM squares.

Current habitats and suggestions for the conservation of the species

The following general statements and suggestions can be made about the examination and evaluation of the Caspian whipsnake habitats.

- In the case of those territories that are not exposed to direct threat it would be necessary to ensure that these areas remain undisturbed. This is possible by surrounding them with a

fence, by heightened monitoring and by limiting their visitability.

- To ensure the conservation of the endangered habitats (Mátyás hill, Szállás hill, Farkas hill, Paks) the habitat management plans must be distinctively tailored to the specific characteristics of these areas.
- It would be necessary to declare legal protection for the directly endangered habitats (Dunaújváros, Dunaföldvár, Dunaszekcső) at the same time when the habitat management plans are elaborated.

Fluctuating asymmetry analysis

Based on the values of the complex indexes and the $FA1_{sublab\ and}$ $FA5_{sublab}$ values, we can compare the state of the Villány Caspian Whipsnake population with the Balaton Dice snake population, the latter living under ideal circumstances. In terms of the frequency of within-population asymmetries no significant difference can be found between the two populations. Based on the fluctuating asymmetry, the Caspian whipsnake population in Villány displays a relatively big developmental stability despite the frequent human disturbance and the occasional habitat destruction. According to this comparison it approximates the values of a snake population with ideal living conditions (BELLAAGH *et al.* 2007B). About the features examined: the discrete value of all the four characters can be registered quickly and easily, even during fieldwork, so according to the special literature (PALMER, 1994) it is ideal for measuring fluctuating asymmetry and therefore their use is highly recommended. In the present analysis the values of two morphological characters did not display an asymmetry with an adequate frequency thus they could not be used when calculating the indexes. In the case of populations exposed to a higher degree of stress these characters, too, are likely to be informative. The other two features displayed a different scale of asymmetry, which confirms the assumption that he flutual asymmetry can be character-specific (CLARKE, 1995).

Habitat preference

According to my results the significance of the individual environmental variables in the habitat choice of the Caspian whipsnake can be different at the level of habitat and region. Certain variables only have a role at the level of habitat; others prove to have a decisive role both at the level of habitat and region. During my examinations it was one element of the landscape, namely

the edge length of the vegetation, which showed a significant difference between the territories used by the snakes and random places at both levels. On these ecoton-like, small territories the so-called high thermal quality habitats, containing several microhabitats with different microclimates, are able to ensure because of their structure both the temperal conditions necessary for the trade-off behaviour of the ectotherm animals and for the active thermoregulation (CARFAGNO *et al.* 2006, ROW, BLOUIN-DEMERS 2006) and the food resources GLANDT 1991, NEMES 2006) on a small territory. It can also be established that both the habitats and the structure of the landscape (the edge-lenghth, the steepness and the mosaicity) have an important influence on the habitat choice of the Caspian whipsnake (BELLAAGH *et al.* 2007A). On a smaller scale a positive correlation was found between the proportion of the free soil and rock surface and the presence of the Caspian whipsnake. This phenomenon calls attention to the structural diversity of the habitats (TEWS *et al.* 2004). The differences in the values of steepness were only significant in the 2x2 meter quadrates and the circles with a 15 meter radius. Therefore, at a small-scale examination the territories demonstrably used by the species (for casting their skin, as habitat or as food resource) proved to be much steeper than the random territories. These territories are much more structured vertically on a small territory scale. According to our current analyses it can be understood that when reconstructing a Caspian whipsnake habitat besides the creation of a diverse microstructure (small-sized rock and soil patches) we need to create a vegetation edge as well (blending a woody and a herbaceous plant surface). Therefore, in all the cases, even the closed woody vegetation, which is native to these areas, should be forced out in patches.

Genetic relationships among the populations in Hungary and abroad, recolonization routes

According to our results we can identify two big haplotype circles on the distribution map of the Caspian whipsnake (NAGY *et al.* 2010). One group includes the populations located on the eastern archipelago of the Aegean Sea and Anatolia. The populations which constitute the other group belong to the European haplotype and are genetically very distinct from the former group. Researches conducted on reptile species with an Eurasian occurrence reveal a similar philogenetical split in the case of the group of *Ablepharus kitaibelii* (POULAKAKIS *et al*. 2005), where an eastern and a eastern subgroup can be well identified within the Eurasian distribution areas. Based on the territorial distribution of the Caspian whipsnake haplotypes, which we

identified during the genetic analyses, we can come to the conclusion that the starting point of the colonisation of the Central-eastern European habitats was the late refuge located on the territory of the Balkan Peninsula. The philogeographical analyses of several reptile species which are present in Eurasia confirmed the fact that the refuges of the Anatolian territories had no significant role in the genesis of the central European populations (JOGER *et al*. 2007). Based on the analyses and the haplotypes-matrix it can be understood that the two most ancient haplotypes are the H5 and H2 haplotypes. The split between the two main haplotypes dates back to a time earlier than the onset of the migration from the western haplotype towards the north. We can find information about the genesis of the Caspian whipsnake populations of the Carpathian basin by examining the populations deriving from the H5 haplotypes and their migration and relationships. Particular attention should be payed to the fact that the H6 haplotype, which only includes samples from Szársomlyó, significantly differs from the quite widespread H5 haplotype, which encompasses habitats from the Sas hill to the Duna estuary and Balkanic habitats. This split from the H5 haplotype is more significant than the split between the H4 and H7 haplotypes, which can be attributed to the accelerated decline of genetic diversity typical of the isolated, island-populations (Szársomlyó H6). Thus, the Caspian whipsnake evidently colonized the Carpathian basin from the south, from the territories south of the Balkan and the line of the Carpathian Mountains. From the current distribution map of the species, according to which the Caspian whipsnake can only be found west of the Danube, we can draw the assumption that the colonisation of the Carpathian basin took place only after the Danube's current flow direction was formed, approximately 10000 years ago (KARÁTSON *et al.* 2006). This theory is confirmed by the fact that in the postglacial era, in the period of the hazel (9000 – 7500 years ago) and the oak (7500 – 5000 years) the Carpathian basin was under the influence of a strong submediterranean climate. In this period steppes were formed on big territories and the hills and mountains were covered by downy oak groves (BORHIDI 1997). Based on the genetic analyses a stronger relationship exists between the populations of the Buda hills and the eastern edge of Mezőföld and those of the Serbian, Romanian, Bulgarian and Macedonian than between the populations of Mezőföld and Szársomlyó. This phenomenon can be attributed to the periodical warming in the last phases of the Pleistocene, during which the Caspian whipsnake extended its are northwards, for the first time at least until it reached the Villány hills. Then due to a wave of colder air - in the period of Beech I and Beech II, 5000-2000 years ago (BORHIDI 1997) - it was

forced to move southwards. The Szársomlyó hill, which stood out from the surroundings as a Mediterranean island even at that time (VARGA 1995) and the favourable climatic conditions of the area helped a small population survive, which still lives in this area. Thus, the Caspian whipsnake could have disappeared from the lower regions during the last, milder wave of cold and then it started re-colonising the Carpathian basin. As it was not possible to gather tissue samples from all the European habitats of the species we cannot exclude the possibility that further independent haplotypes can be found within the territory of the H5 haplotype, which have a genetic disposition similar to that of the H6 haplotype. This would support the gradual northwards radiation of the species. In so far as we accept the abovementioned colonisation theory it also becomes evident that the genetic background of the fluctuating asymmetry is quite low; as the frequency of fluctuating asymmetry in the Szársomlyó population, which has been demonstrably isolated for a long time, is not significantly higher than in the case of a dice snake population, which is situated on a coherent area and boasts ideal living conditions as well as a high number of specimen.

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RELATRED PUBLICATIONS

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*Communications indicated with an * do not appear in the bibliography in order to avoid repetitions.*