

When turtle distribution tells European history: mtDNA haplotypes of *Emys orbicularis* reflect in Germany former division by the Iron Curtain

Uwe FRITZ¹, Daniela GUICKING², Peter LENK³, Ulrich JOGER⁴
& Michael WINK²

¹Museum of Zoology, Natural History State Collections Dresden, A. B. Meyer Building, Königsbrücker Landstr. 159, D-01109 Dresden, Germany; e-mail: uwe.fritz@snsd.smwk.sachsen.de

²Institute of Pharmacy and Molecular Biotechnology (IPMB), Biological Department, Heidelberg University, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany

³Seestr. 6a, D-63796 Kahl am Main, Germany

⁴Staatliches Naturhistorisches Museum, Pockelsstr. 10, D-38106 Braunschweig, Germany

FRITZ, U., GUICKING, D., LENK, P., JOGER, U. & WINK, M., When turtle distribution tells European history: mtDNA haplotypes of *Emys orbicularis* reflect in Germany former division by the Iron Curtain. *Biologia, Bratislava*, 59/Suppl. 14: 19–25, 2004; ISSN 0006-3088.

The mitochondrial DNA haplotypes of 75 wild caught *Emys orbicularis* from Germany have been determined. Eleven different haplotypes belonging to six clades were identified. Only turtles bearing a haplotype endemic to eastern Germany and neighbouring western Poland are considered to be native. They are restricted today to the German federal states of Mecklenburg-Western Pomerania and Brandenburg. An individual of a recently exterminated Saxon population bears this haplotype, too. The majority of German turtles exhibit other haplotypes from different parts of the species' range. The haplotype distribution reflects the previous division of Germany by the Iron Curtain. In former East Germany, haplotypes from East Block countries accessible to GDR citizens predominate; whereas in former West Germany a greater variety of haplotypes was found. This mirrors the greater extent of the West German pet trade and, surely, also the destinations of countless East and West German tourists in Southeast Europe and the Mediterranean. Some turtles in East and West Germany bear a haplotype naturally occurring in former parts of Germany now belonging to Poland and Russia. They could either represent introductions from these areas and adjacent eastern countries prior to World War II or recently smuggled individuals from Poland or the former USSR.

Key words: *Emys orbicularis*, Emydidae, genetics, mtDNA, haplotype, conservation, Germany.

Introduction

Recent years have seen a rapid increase in our understanding of the systematics and phylogeog-

raphy of the European pond turtle, *Emys orbicularis* (L., 1758). This species is distributed from North Africa over most of Europe south of Scandinavia and Asia Minor to the Caspian and

Aral Seas (FRITZ, 2001, 2003). *E. orbicularis* was thought to be a text-book example of a wide-ranging, monotypic species for dozens of years. However, recent studies, as reviewed in FRITZ (2001, 2003), have shown that *E. orbicularis* is one of the most fragmented reptilian taxa of the western Palearctic. Currently, 13 morphologically distinctive subspecies are recognized and some taxa are still awaiting their description (FRITZ, 2001, 2003). A phylogeographic investigation using mainly the mitochondrial cytochrome *b* gene revealed the existence of 20 different haplotypes, divided into seven distinct clades (LENK et al., 1999), which largely correlate, as far as studied, with the subspecies (FRITZ, 2001, 2003). In Germany, genetic haplotyping combined with an analysis of morphological characters has been used to find out whether captured specimens are native or not (KUPRIAN et al., 2001; SCHNEEWEISS, 2003). Here we review the distribution of *E. orbicularis* haplotypes in Germany and re-evaluate previous interpretations of these data relating to the provenance of putative native turtles.

Material and methods

In the course of our ongoing studies on the phylogeography of *Emys orbicularis*, we obtained mtDNA sequence data of approximately 950 blood and tissue samples from most of the species' range. Still lacking or underrepresented are data from North Africa, parts of the Iberian peninsula, Turkey, Iran, and Turkmenistan. From Germany we analyzed 75 samples, originating from wild pond turtles from the federal states of Baden-Wuerttemberg (3), Bavaria (1), Brandenburg (23), Hamburg (1), Hesse (18), Lower Saxony (15), Mecklenburg-Western Pomerania (1), North Rhine-Westphalia (4), Saxony (5), Saxony-Anhalt (3), and Schleswig-Holstein (1). Most turtles have been captured between 1994 and 2003 by local nature conservation authorities and were released after blood sampling. Some roadkills and drowned specimens from fishtraps are now in the collection of the Museum of Zoology Dresden.

Blood or muscle tissues were taken and stored as described in HASKELL & POKRAS (1994) and ARCTANDER (1988). Total genomic DNA was extracted following standard proteinase K and phenol-chloroform protocols (SAMBROOK et al., 1989). PCR and sequencing are explained in detail in LENK et al. (1999). We define haplotypes and haplotype clades according to individual mtDNA sequences (LENK et al., 1999). Our target sequence is the mitochondrial cytochrome *b* gene. Of the 1031 aligned sites, 69 are variable. 63 substitutions are transitions, and six are transversions; 41 sites are parsimony informative. 13 sites are variable at the first, eight at the second, and 48 at the third codon position. For each sequence, variable sites are checked individually to prevent errors from incorrect

sequencer output. EMBL accession numbers for sequences are: AJ131407-131426, AY652865-AY652884, and AY652887-AY652890.

We use a minimum spanning network calculated with the program Arlequin (SCHNEIDER et al., 2000) to illustrate that the haplotypes found in Germany are very distinct and often not closely related, suggesting multiple geographic origins (Fig. 1). In this network presentation, each haplotype is connected to all other haplotypes so that the overall number of assumed mutations is minimized. On the infraspecific level, a network is superior to a dichotomous tree because it is able to demonstrate simultaneously alternative evolutionary pathways. Intraspecific population genealogies are often multifurcated and descendant genes may coexist with persisting ancestors. A network allows for persistent ancestral nodes and reticulations. The occurrence of reticulations visualizes ambiguous or uncertain domains. In haplotypic data, loops may indicate the occurrence of reverse or parallel mutations (POSADA & CRANDALL, 2001).

Results

In an earlier study we identified 20 mtDNA haplotypes in *Emys orbicularis*, representing seven distinct clades (LENK et al., 1999). Since then, we found 24 additional haplotypes and two new clades, raising the number of known haplotypes considerably to 44 and the number of clades to nine (Fig. 1). One new haplotype, corresponding to a distinct new clade (VIIIa), was discovered in southern Turkey. The other new clade, also represented by a newly identified haplotype (IXa), was found in a captive turtle lacking locality data.

Our new data corroborate and refine the general picture already outlined in LENK et al. (1999) (Fig. 2). Most of the haploclades are restricted to the southern parts of the species' range, arguing for a postglacial recolonization of the more northerly regions from only very few glacial refugia (FRITZ, 1996, 2003; LENK et al., 1999). Among our 75 samples from Germany, we detected eleven different haplotypes belonging to six clades (lineages I, II, III, IV, V, VI of LENK et al., 1999). Some of the identified haplotypes, differing only in a few mutations from others, possess very restricted native ranges, enabling a quite exact allocation of the geographic origin of the turtles (Tab. 1).

Only one haplotype, IIb, which is endemic in northeastern Germany and neighbouring Poland, represents unambiguously native German pond turtles. All other haplotypes correspond to individuals that have been introduced intentionally or escaped from captivity. Interestingly, the haplotype distribution reflects the previous political division of Germany (Fig. 3): The haplotypes of

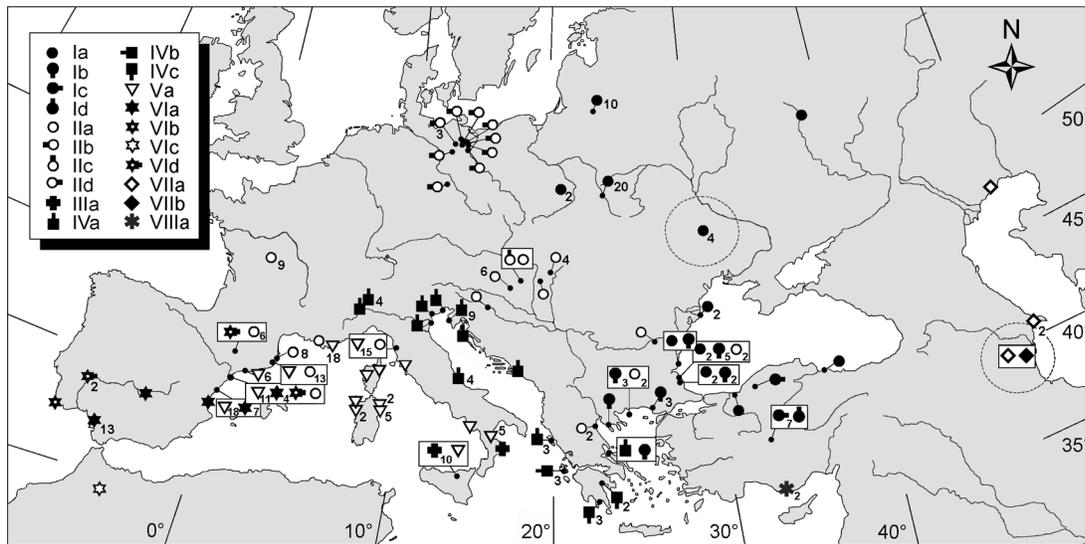


Fig. 2. Geographic distribution of 316 mtDNA haplotypes in native *Emys orbicularis*. Numbers by the symbols indicate frequencies of haplotypes per locality. Symbols without numbers represent single specimens, symbols in boxes mark polymorphic populations, dashed circles denote inexact localities. Haplotype symbols are explained in the box. Haplotype IXa (not shown) was found until now only in a sample of a pet trade turtle lacking locality data. Modified from LENK et al. (1999).

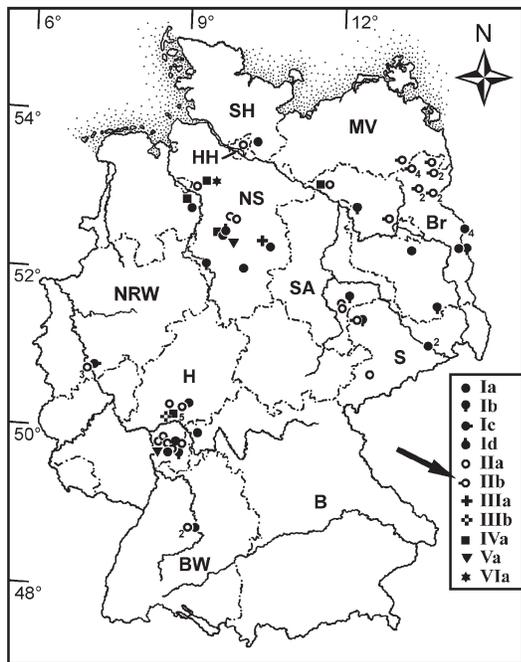


Fig. 3. Geographic distribution of 75 mtDNA haplotypes of *Emys orbicularis* in Germany. The native haplotype IIb is indicated by an arrow. Dashed lines designate borders of federal states. B – Bavaria (Bayern), Br – Brandenburg, BW – Baden-Wuerttemberg, H – Hesse (Hessen), HH – Hamburg, MV – Mecklenburg-Western Pomerania (Mecklenburg-Vorpommern), NRW – North Rhine-Westphalia (Nordrhein-Westfalen), NS – Lower Saxony (Niedersachsen), S – Saxony (Sachsen), SA – Saxony-Anhalt (Sachsen-Anhalt), SH – Schleswig-Holstein. The former German Democratic Republic (GDR) comprised Brandenburg, Mecklenburg-Western Pomerania, Saxony, Saxony-Anhalt, and Thuringia (no specimens studied). For further explanations see Figure 2.

They are huge and very dark coloured, as characteristic for pond turtles of northern populations (FRITZ, 2001, 2003). These turtles could represent

old introductions from former parts of Germany belonging now to Poland and Russia (e. g. West and East Prussia) or from adjacent countries. It

is known that pond turtles from Poland, Russia, and Ukraine have been released in different parts of Germany prior to World War II (FRITZ, 2001, 2003; SCHNEEWEISS, 2003). Otherwise, some haplotype Ia turtles could have been recently smuggled from Poland or the former USSR. This seems likely for recently captured individuals from degraded water bodies in the outskirts of cities (e. g. two turtles from the suburbs of Dresden, collected in 2001 and 2003). In former East Germany only one turtle was found with a haplotype not known from former East Block countries. It is an individual with haplotype IVa from westernmost Brandenburg. FRIEDEL (1868) reported of repeated efforts to introduce during the 19th century turtles from the Bay of Venice (Italy) in Brandenburg. The Bay of Venice is an area where haplotype IVa turtles occur (LENK et al., 1999). Our specimen is perhaps derived from such a historic introduction.

Discussion

The difference between Brandenburg and Hesse
Emys orbicularis is currently widespread in Germany. Records exist for nearly the whole country. However, the majority of records refer to introduced pond turtles or escaped pets (FRITZ & GÜNTHER, 1996; PODLOUCKY, 1997). Most authors accept that native populations are restricted today to the federal states of Brandenburg and Mecklenburg-Western Pomerania, the two German states with the lowest human population density (88 and 75 people per square kilometre; STATISTISCHES BUNDESAMT DEUTSCHLAND, 2004). In the adjacent state Saxony, a native pond turtle population was exterminated by brown-coal mining in the second half of the 20th century (FRITZ, 2001, 2003). Until today, a single male of that population is kept in captivity, which was included into our study. Nowadays, the relict populations of *E. orbicularis* comprise at best 10–15 turtles in Brandenburg and Mecklenburg-Western Pomerania. Often only single individuals are found there (FRITZ & GÜNTHER, 1996; SCHNEEWEISS & FRITZ, 2000; FRITZ, 2001, 2003; SCHNEEWEISS, 2003). For Brandenburg and Mecklenburg-Western Pomerania, the continuous occurrence until now is well documented by subfossil findings, many historic sources, and literature records (DÜRIGEN, 1897; FRIEDERICH, 1910; SCHNEEWEISS, 1997, 2003; SCHNEEWEISS & FRITZ, 2000; FRITZ, 2003).

Recently, KUPRIAN et al. (2001) claimed that native, relict populations also occur in the

south of the federal state of Hesse. In contrast to Brandenburg and Mecklenburg-Western Pomerania, Hesse belongs to the most densely populated regions of Germany (288 people per square kilometre; STATISTISCHES BUNDESAMT DEUTSCHLAND, 2004), and this is especially true for southern Hesse (497 people per square kilometre; ANONYMOUS, 2004). In southern Hesse, also exotic turtles (*Chinemys*, *Pseudemys*, *Trachemys*) are frequently found (NESEMANN, 1986, 1987; HANKA & JOGER, 1998), and there is no clear evidence for the occurrence of pond turtles in historic times. However, subfossil findings are known there, and from nearby South German regions records for *E. orbicularis* exist until the beginning of the 18th century (KINZELBACH, 1988; FRITZ, 2001, 2003).

In both areas, northeastern Germany (Brandenburg, Mecklenburg-Western Pomerania; SCHNEEWEISS, 2003) and southwestern Germany (Hesse; KUPRIAN et al., 2001), the occurrence of a certain mtDNA haplotype has been used to assess whether a given specimen is native or not. Native turtles from northeastern Germany resemble individuals of the same subspecies (*Emys orbicularis orbicularis*) from eastern Poland (FARKAS et al., 1998) and other countries morphologically but bear a distinct mtDNA haplotype (LENK et al., 1999). This haplotype (IIb) is endemic to northeastern Germany and neighbouring western Poland (LENK et al., 1999 and unpubl.; Fig. 2, Tab. 1). Hence, it is likely that any pond turtle from northeastern Germany bearing this haplotype is native. Also the mentioned male from Saxony possesses this haplotype. However, it is important to keep in mind that the autonomous mitochondrial DNA (mtDNA) is not part of the actual (chromosomal) genome. Moreover, mtDNA is inherited exclusively in the maternal line and does not recombine with the parental mtDNA. As a consequence, specimens with haplotype IIb, might be also the result of a mating of a native female with an allochthonous male. Also back-crosses and later hybrid generations bear solely the mitochondrial haplotype of the maternal ancestor and cannot be identified as hybrids by mitochondrial haplotyping.

In Hesse, the occurrence of the closely related haplotype IIa has been taken as evidence for autochthony (KUPRIAN et al., 2001). However, compared with IIb, haplotype IIa has a distinctly wider natural distribution (Fig. 2, Tab. 1). It was found from the northern Spanish Mediterranean coast to the lower course of the Danube river and the southeastern Balkans. In west-east direction,

the distribution of haplotype IIa covers approximately 2000 km.

Subfossil findings and historic sources document that southern Germany and adjacent Central Europe were inhabited by native pond turtles in the Holocene. However, native South German, Czech, and Swiss populations are thought to be extinct (FRITZ, 1996, 2001, 2003; ŠIROKÝ, 2000). They fill the recent gap between the French and eastern Central European range of haplotype IIa turtles. Therefore, the native pond turtles once inhabiting southern Germany (and adjacent countries) should have had the same mitochondrial haplotype. Nevertheless, the occurrence of haplotype IIa in an individual found there today is not compelling evidence for autochthony. Until the trade with European pond turtles ceased in West Germany with the implementation of the federal species protection order in 1980, a major source for pet turtles was the Danube plain in former Yugoslavia. This is an area where haplotype IIa turtles occur.

The last records of unambiguously native pond turtles in South Germany date back to the early 18th century (FRITZ, 2001, 2003). In a densely populated region like southern Hesse it is most unlikely that *E. orbicularis* populations could exist for centuries without having been detected. Thus, we suppose that the Hessian *E. orbicularis* with haplotype IIa are recently escaped or intentionally introduced pet turtles.

In conclusion, mtDNA haplotypes are a powerful tool for discriminating native and introduced individuals if the haplotypes have a very restricted geographical range. This is the case with haplotype IIb, which occurs exclusively in western Poland and northeastern Germany. However, even a turtle bearing the correct haplotype can be the result of hybridization with an introduced specimen. If mtDNA haplotypes occur in vast areas, there is no convincing evidence that a given specimen originates in a certain part of the distribution area of the haplotype.

Acknowledgements

Many thanks go to F. BEHREND, G. DAMER, T. DERNBACH, W.-R. GROSSE, R. PODLOUCKY, F. PRELLWITZ, N. SCHNEEWEISS, R. STAMPFER, J. UNVERFÄRTH, and R. WICKER, who provided blood samples or dead turtles for this study. J. F. PARHAM and M. VENCES reviewed the manuscript.

References

- ANONYMOUS. 2004. Hessen. Zahlen aus Hessen. <http://www.hessen.de>.
- ARCTANDER, P. 1988. Comparative studies of Avian DNA by restriction fragment polymorphism analysis. *J. Ornithol.* **129**: 205–216.
- DÜRIGEN, B. 1897. Deutschlands Amphibien und Reptilien. Creutz, Magdeburg, VIII, 676 pp., 12 pls.
- FARKAS, B., FRITZ, U., JENDRETZKE, N. & SCHNEEWEISS, N. 1998. Morphological differences between pond turtles (*Emys orbicularis*) from the Hungarian Lowlands, eastern Poland, and northeastern Germany, pp. 89–94. In: FRITZ, U., JOGER, U., PODLOUCKY, R. & SERVAN, J. (eds) Proceedings of the EMYS Symposium Dresden 96, Mertensiella **10**.
- FRIEDEL, E. 1868. Neues über Züchtung und Eingewöhnung der Auster. II. Eingewöhnung. *Zool. Garten* **9**: 298–304.
- FRIEDERICHS, K. 1910. Ueber die Verbreitung der Sumpfschildkröte (*Emys orbicularis* L.) in der Provinz Brandenburg und in Mecklenburg-Strelitz. *Mitt. Fischerei-Ver. Brandenburg* **2**: 201–208, 243.
- FRITZ, U. 1996. Zur innerartlichen Variabilität von *Emys orbicularis* (Linnaeus, 1758). 5b. Intraspezifische Hierarchie und Zoogeographie. *Zool. Abh. Mus. Tierkd. Dresden* **49**: 31–71.
- FRITZ, U. 2001. *Emys orbicularis* (Linnaeus, 1758) – Europäische Sumpfschildkröte, pp. 343–515. In: FRITZ, U. (ed.) Handbuch der Reptilien und Amphibien Europas, Band 3/IIIA: Schildkröten I, Aula, Wiebelsheim.
- FRITZ, U. 2003. Die Europäische Sumpfschildkröte. Laurenti, Bielefeld, 224 pp.
- FRITZ, U. & GÜNTHER, R. 1996. Europäische Sumpfschildkröte – *Emys orbicularis* (Linnaeus, 1758), pp. 518–534. In: GÜNTHER, R. (ed.) Die Amphibien und Reptilien Deutschlands, Fischer, Jena.
- HANKA, S. & JOGER, U. 1998. *Emys orbicularis* in the Enkheimer Ried near Frankfurt/Main, Hesse, pp. 135–140. In: FRITZ, U., JOGER, U., PODLOUCKY, R. & SERVAN, J. (eds) Proceedings of the EMYS Symposium Dresden 96, Mertensiella **10**.
- HASKELL, A. & POKRAS, M. A. 1994. Nonlethal blood and muscle tissue collection from redbelly turtles for genetic studies. *Herpetol. Rev.* **25**: 11–12.
- KINZELBACH, R. 1988. Die Europäische Sumpfschildkröte (*Emys orbicularis*) im Einzugsgebiet des Rheins. *Zeitschr. Angew. Zool.* **75**: 385–419.
- KUPRIAN, M., WINKEL, S., HOMEIER, O., HOMEIER, M., KLEIN, H.-J., MÄHN, M. & WICKER, R. 2001. Reinheimer Sumpfschildkröten erhalten hessischen Pass. Molekularbiologische Untersuchungen bringen Klarheit. *Jahrb. Natursch. Hessen* **6**: 239–240.
- LENK, P., FRITZ, U., JOGER, U. & WINK, M. 1999. Mitochondrial phylogeography of the European pond turtle, *Emys orbicularis* (Linnaeus 1758). *Mol. Ecol.* **8**: 1911–1922.

- NESEMANN, H. 1986. Die Wasserschildkröten in der Untermainaue im Jahre 1983. Hess. Faunist. Briefe **6**: 68–70.
- NESEMANN, H. 1987. Berichtigung und Ergänzung zu “Die Wasserschildkröten in der Untermainaue im Jahre 1983“. Hess. Faunist. Briefe **7**: 18.
- PODLOUCKY, R. 1997. *Emys orbicularis* (Linnaeus, 1758), pp. 170–171. In: GASC, J.-P. et al. (eds) Atlas of Amphibians and Reptiles in Europe, Societas Europaea Herpetologica and Muséum National d’Histoire Naturelle, Paris.
- POSADA, D. & CRANDALL, K. A. 2001. Intraspecific gene genealogies: trees grafting into networks. Trends Ecol. Evol. **16**: 37–45.
- SAMBROOK, J., FRITSCH, E. F. & MANIATIS, T. 1989. Molecular Cloning: a Laboratory Manual. 2nd ed. Cold Spring Harbor, N. Y., Cold Spring Harbor Laboratory Press, 1659 pp.
- SCHNEEWEISS, N. 1997. Fang, Handel und Aussetzung – historische und aktuelle Aspekte des Rückgangs der Europäischen Sumpfschildkröte (*Emys orbicularis* Linnaeus, 1758) in Brandenburg. Natursch. Landschaftspfl. Brandenburg **1997**: 76–81.
- SCHNEEWEISS, N. 2003. Demographie und ökologische Situation der Arealrand-Populationen der Europäischen Sumpfschildkröte in Brandenburg. Studien Tagungsber., Landesumweltamt Brandenburg **46**: 1–106.
- SCHNEEWEISS, N. & FRITZ, U. 2000. Situation, Gefährdung und Schutz von *Emys orbicularis* (L.) in Deutschland, pp. 133–144. In: HÖDL, W. & RÖSSLER, M. (eds) Die Europäische Sumpfschildkröte, Stapfia **69**.
- SCHNEIDER, S., ROESSLI, D. & EXCOFFIER, L. 2000. Arlequin ver. 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- ŠIROKÝ, P. 2000. A review of the distribution of the European pond turtle, *Emys orbicularis* (Linnaeus, 1758) in the Czech Republic until 1999. Faunist. Abh. Mus. Tierkd. Dresden **22**: 69–83.
- STATISTISCHES BUNDESAMT DEUTSCHLAND. 2004. Deutschland nach Ländern. Gebiet und Bevölkerung. <http://www.destatis.de>.