Revista Española de Herpetología

IV INTERNATIONAL SYMPOSIUM ON EMYS ORBICULARIS

Asociación Herpetológica Española
Volumen 22 (2008)
VALENCIA

Rev. Esp. Herp. (2008) 22:5-14

High prevalence of accessory scutes and anomalies in Iberian populations of *Emys orbicularis*

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Abstract: In a previous paper we found that most individuals from two populations of *E. orbicularis* from NW Spain had accessory scutes and other anomalies in their carapace. In this paper we analyze 10 additional populations [Ribadavia, NW Spain, Boticas (N Portugal), Castro Verde and Almograve (S Portugal), Salamanca and Zamora (Central-West Spain), Madrid (Central Spain), Ciudad Real (Central-Southern Spain), Doñana National Park (SW Spain) and Valencia (E Spain)] and increase previous samples from NW Spain. We found that the proportion of anomalous specimens oscillates between 3% in Doñana and 69% in Porriño. The incidence of anomalies varies significantly between populations, and there is a tendency for an increase of these anomalies from South to North. Non-Iberian populations of *E. orbicularis* rarely show anomalous individuals (except for some areas of Poland and Belarus). In other turtle species these anomalies seem to be due mainly to suboptimal humidity and temperature conditions during incubation. We suggest that the high prevalence of anomalies in Iberian populations of *E. orbicularis* might be due to a genetic predisposition of Iberian lineages, but other explanations based on the effects of pollution and problems due to the loss of genetic variability in Northern populations might also contribute to the frequency of this trait.

Key words: *Emys orbicularis*, Iberian peninsula, scute anomalies.

Resumen: Elevada presencia de anomalías y placas extra en poblaciones ibéricas de *Emys orbicularis*. – En un trabajo previo encontramos que la mayoría de los individuos de dos poblaciones de *E. orbicularis* del NO de la península Ibérica tenían escudos extra y otras anomalías en el caparazón. Mediante el análisis de muestras de 10 poblaciones adicionales [Ribadavia, NO de España, Boticas (N Portugal), Castro Verde y Almograve (S Portugal), Salamanca y Zamora (Centro-Oeste de España), Madrid (Centro de España), Ciudad Real (Centro-Sur de España), Parque Nacional de Doñana (SO de España) y Valencia (E de España)] y el incremento de las muestras de las poblaciones del NO, encontramos que el porcentaje de individuos anómalos oscila entre el 3% de Doñana y el 69% de Porriño. El porcentaje de individuos anómalos varía significativamente entre poblaciones, y existe una tendencia que sugiere un incremento de las anomalías hacia el norte. Una revisión de la bibliografía indica que las poblaciones no Ibéricas de *E. orbicularis* presentan raramente este tipo de anomalías (con la excepción de algunas poblaciones de Polonia y Bielorrusia). En otras especies de tortugas los individuos anómalos parecen ser debidos fundamentalmente a problemas de humedad y/o temperatura durante la incubación. Se sugiere que las anomalías de las poblaciones Ibéricas de *E. orbicularis* pudieran ser propias de los linajes de la península, aunque otras explicaciones basadas en el efecto de la contaminación y problemas derivados de la pérdida de variabilidad genética podrían también contribuir a su formación.

Palabras clave: anomalías en el caparazón, *Emys orbicularis,* Península Ibérica.

INTRODUCTION

In recent years there has been a growing interest in phenotypic plasticity and its relation to fitness (PIGLIUCCI, 2001). It is generally assumed that when organisms are under suboptimal conditions they might develop abnormally, because their buffer ability might be exceeded. For instance, traits that are expressed in both parts of bilateral organisms, might develop in a slightly different way, and if population left-right differences follow a normal distribution with a mean of zero, then the trait is said to show fluctuating asymmetry (PALMER & STROBECK, 1986). In extreme cases, organisms might show abnormalities that change their phenotype so drastically that their general appearance might be described as teratologic. For instance, embryos of the freshwater turtle *Chelydra serpentina* from Ontario (Canada) sometimes develop without tail, legs or claws (BISHOP *et al.*, 1991), and there is good evidence that organochloride contamination is the main cause of these anomalies (BISHOP *et al.*, 1998) and other morphological changes in hatchlings (BISHOP *et al.*, 1994; de SOLLA *et al.*, 1998). This link between pollution (or other stress agents) and morphology of indicator organisms has been used to monitor habitat quality in freshwater ecosystems (HARDERSEN, 2000; NUNES *et al.*, 2001).

In 1996 we started a long-term study of a population of the European pond turtle (*Emys orbicularis*) in NW Spain. To our surprise most individuals (75%) showed conspicuous carapacial scute anomalies, including accessory scutes, lack of scutes and big asymmetries (AYRES FERNÁNDEZ & CORDERO RIVERA, 2001). Given that this population is found in a river basin with extreme industrial and urban pollution (ALVAREZ-CAMPANA GALLO, 1996), we hypothesized that pollution might be the cause of the anomalies. Nevertheless, in late 2001 we found a second population in a rural area far from pollution sources that also showed a surprisingly high proportion of anomalous individuals (40%) (AYRES FERNÁNDEZ & CORDERO RIVERA, 2004). This led us to develop a research program to study these anomalies in Iberian populations.

At least three hypotheses could explain this high incidence of anomalies in NW Spain turtles: (1) the region is in a suboptimal climatic area, and eggs incubated under suboptimal conditions of temperature and humidity are more likely to develop into anomalous or less fit specimens (LYNN & ULLRICH, 1950; PAVALKO, 1986; BOBYN & BROOKS, 1994; JUDGE, 2001; KAZMAIER & ROBEL, 2001; ASHMORE & JANZEN, 2003; HERLANDS *et al.*, 2004), (2) anomalies could be produced by exposure to chemicals, and therefore be attributed to pollution (BISHOP *et al.*, 1991, 1998), and (3) these Northern isolated populations may have lost most of its genetic variability and this causes the observed anomalies (NAJBAR & MACIANTOWICZ, 2000). All three hypotheses could jointly explain the observed anomalies, because they are not incompatible.

In this paper we present an overview of the incidence of scute anomalies in Iberian populations of *E. orbicularis*. During 2003-2005 we sampled several Iberian populations, in Spain and Portugal, to obtain blood samples for further genetic diversity analysis, take biometrical measures and examine individuals for anomalies.

MATERIAL AND METHODS

Samples of individuals of *E. orbicularis* were obtained from 12 populations (Table 1) including Northern, Central and Mediterranean populations. Individuals (N = 531) were captured with baited traps, measured

TABLE 1. The number of individuals (N) of *Emys orbicularis* from Iberian populations showing a different number of carapace anomalies, the mean number of anomalies per individual and the proportion of anomalous specimens. P = Portuguese localities.

TABLA 1. Número de individuos (N) de *Emys orbicularis* de poblaciones ibéricas que presentan diferentes tipos de anomalías, con el número medio de anomalías por individuo, y la proporción de individuos anómalos. P = localidades portuguesas.

Population	Latitude	Number of anomalies						Mean	% anomalous	N
		0	1	2	3	4	5			
Doñana (Huelva)	37.26	33	1	0	0	0	0	0.03	0.03	34
Almograve [P]	37.65	12	6	4	4	0	0	1.00	0.54	26
Castroverde [P]	37.70	8	3	0	0	0	0	0.27	0.27	11
Ciudad Real	38.98	16	5	2	0	0	0	0.39	0.30	23
Valencia	39.47	39	11	5	0	0	0	0.38	0.29	55
Madrid	40.40	29	2	1	0	0	0	0.13	0.09	32
Salamanca	40.60	6	0	1	0	0	0	0.29	0.14	7
Zamora	41.32	13	4	0	1	0	0	0.39	0.28	18
Boticas [P]	41.68	27	5	8	1	0	1	0.69	0.36	42
Porriño (Pontevedra)	42.17	52	45	38	25	3	3	1.34	0.69	166
Río Arnoia (Ourense)	42.18	46	16	10	1	1	0	0.58	0.38	74
Ribadavia (Ourense)	42.28	16	12	7	5	3	0	1.23	0.63	43

and photographed and, for most of them, a sample of blood was obtained for further genetic analyses. In most localities, all individuals were captured at 1-3 nearby rivers or ponds, except in Valencia, where data were obtained at two ponds and also from animals in a Recovery centre. Therefore, in this last case some caution is needed when interpreting the results because animals of unknown origin are included in the sample. Furthermore genetic evidence showed the presence of at least two different lineages among Valencian specimens (VELO-ANTÓN *et al.*, 2008).

We considered as anomalous any individual showing at least one accessory scute on the carapace or plastron or the lack of at least one scute (see AYRES FERNÁNDEZ & CORDERO RIVERA, 2004, for examples of some of these anomalies). Small asymmetries in the morphology of the scutes were not taken into account. The full list of anomalies is presented in Table 2.

To test for an association between latitude and proportion of anomalous individuals, we used a GLM with binomial errors and logit link, corrected for overdispersion. The response variable was the number of anomalous individuals, and the binomial total the sample size for each population. To test for sexual differences in anomalies, we performed a GLM with Poisson error distribution, using sex and population as factors, and the number of anomalies per individual as the response variable. Statistical analyses were done with Genstat 10th edition (GENSTAT, 2007).

RESULTS

Table 1 summarizes the proportion of anomalous turtles in different populations, and these are presented in Fig. 1. Table 2 shows the number of animals with each anomaly. The most common anomaly is an accessory scute between vertebrals 4th and

TABLE 2. Distribution of anomalies per population. Absolute frequencies of individuals showing each kind of anomaly (individuals with more than one anomaly are counted in each column). The column total indicates how many individuals show each anomaly from a total sample of 531 (including adults, juveniles and hatchlings). P = pleural scute, V = vertebral scute.

TABLA 2. Distribución de anomalías por población. Frecuencias absolutas de individuos que presentan cada tipo de anomalía (aquellos individuos con más de una anomalía se cuentan en cada columna). La columna Total indica cuantos individuos muestran cada anomalía de una muestra total de 531 individuos (incluyendo adultos, juveniles y neonatos). P = escudo pleural, V = escudo vertebral.

Population	Accesory scute left P1-V1	Accesory scute right P1-V1	Accesory scute V1/V2	Accessory scute V2/V3	Accessory scute V3/V4	Accessory scute V4/V5	Vertebral divided	Interior scute	Accessory pleural scute	Lack of pleural scute	Nuchal divided	Accessory marginal scute	Accessory gular scute	Accessory scute Gul/Hum	Accessory scute Hum/Pec	Accessory scute Abd/Fem	Accessory scute Fem/Anal	Plastron divided
Doñana (Huelva)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Almograve	9	7	0	0	4	2	0	1	0	0	0	0	0	2	1	0	0	0
Castro Verde	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2
Ciudad Real	1	0	0	0	0	4	3	0	0	0	0	0	0	0	0	1	0	0
Valencia	4	1	0	0	1	6	2	1	2	0	1	3	0	0	0	0	0	0
Madrid	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0
Salamanca	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Zamora	2	1	0	0	0	1	0	0	2	0	0	0	0	1	0	0	0	0
Boticas	7	11	0	1	1	2	2	3	1	0	0	0	0	0	0	1	0	0
Porriño (Pontevedra)	43	42	0	1	5	77	17	20	7	2	0	0	6	2	1	0	0	0
Río Arnoia (Ourense)	10	7	1	0	1	10	1	5	4	0	0	0	1	0	0	1	1	1
Ribadavia (Ourense)	6	9	3	2	6	23	2	0	0	0	0	0	0	0	0	0	0	2
Total	83	80	4	4	18	127	27	30	16	2	1	4	7	5	2	5	1	5

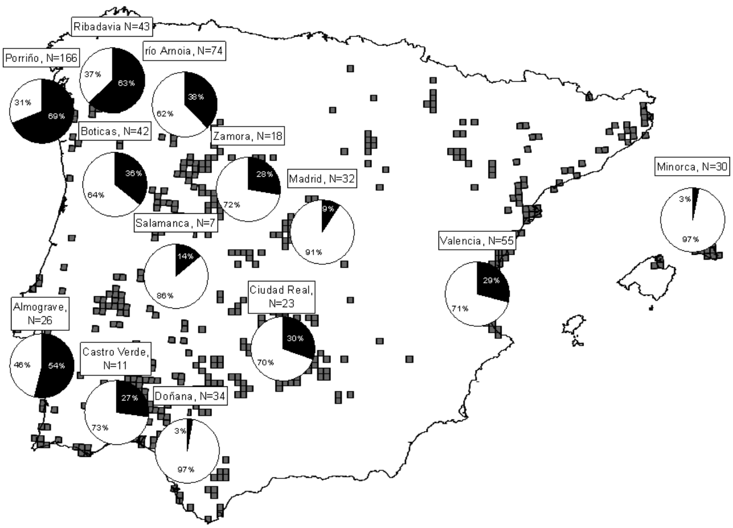

FIGURE 1. The proportion of anomalous specimens (closed sectors) in Iberian populations of *E. orbicularis*, overlaid on a map of the distribution of the species. Data from Minorca are based on VEYSSET (2005).

FIGURA 1. Proporción de individuos anómalos en poblaciones ibéricas de *E. orbicularis*, sobre un mapa de la distribución de la especie. Los datos de Menorca están basados en VEYSSET (2005).

5th, present in 54% of individuals with at least one anomaly (in 24% of all individuals), followed by accessory scutes between left 1st pleural and 1st vertebral (35 and 16%) and right 1st pleural and 1st vertebral (34 and 15%).

The proportion of anomalous animals increases from South to North (Fig. 1; GLM with binomial errors corrected for overdispersion, deviance ratio = 6.42, $p = 0.030$). When including in this analysis the proportion of anomalies in Minorca (VEYSSET, 2005) and the data for Zamora with a larger sample size (ALARCOS *et al.*, 2005) (Table 2), there is no substantial change in the result (deviance ratio = 5.17, $p = 0.044$). Three populations have surprisingly high proportion of anomalous individuals: Porriño (69%), Ribadavia (63%) and Almograve (54%). These populations have also a high proportion of individuals showing more than one anomaly (Tables 1 and 2).

To test for sexual differences in the number of anomalies per individual, we entered Population and Sex as explanatory variables in a GLM with Poisson errors. The model is significant (deviance ratio = 5.30, $p < 0.001$), but this is due only to differences among populations, because the effect of Sex is not significant (t-test: $t_{360} = 0.94$, $p = 0.345$). This is confirmed by a model with only Sex as explanatory variable, which is not significant (deviance ratio = 0.04, $p = 0.846$).

DISCUSSION

In a previous paper we reported the high incidence of anomalies in turtles from Porriño and Ourense populations (NW Spain) (AYRES FERNÁNDEZ & CORDERO RIVERA, 2004). The results of this survey indicate that the presence of accessory scutes and other shell anomalies is a common fact in Iberian populations of *E. orbicularis*. Scute anomalies are found probably in all populations of *E. orbicularis*. For instance, in his classic work ROLLINAT (1934) reports several individuals with one or more accessory scutes, but unfortunately does not indicate the proportion of animals showing these anomalies. Nevertheless they seem to be rare in non-Iberian *E. orbicularis* populations (CHEREPANOV, 1994; SCHNEEWEISS & BECKMANN, 2005), with the exception of Poland (NAJBAR & MACIANTOWICZ, 2000; NAJBAR & SZUSZKIEWICZ, 2005), Belarus (DROBENKOV, 2005) and Switzerland (MOSIMANN, 2002). In the last case this could be due to the allocthonous origin of part of the population, and represent a case of outbreeding depression.

In his review of the turtle shell, ZANGERL (1969) found that accessory or asymmetric scutes occur in approximately 15% of individuals of many turtle species. A review of the literature indicates that such anomalies are widespread, or at least are reported frequently in recent years (Table 3). It is likely that when scute anomalies are rare (say less than 5% of individuals), can remain unreported because might be considered anecdotal. Therefore we suggest that the values compiled in Table 3 are biased to populations with high frequencies of anomalous specimens. Even taking into account this consideration, our data indicates that Iberian populations of *E. orbicularis* are highly anomalous.

To test the hypotheses that were put forward and listed in the Introduction section, an experimental study is needed. If the anomalies are due to pollution, then we can predict that their incidence should be very low in pristine areas. Evidence in favour of this hypothesis is the high prevalence of anomalies in Porriño, an area with urban and industrial pollution (including the organochloride lindane) (ALVAREZ-CAMPANA

TABLE 3. Scute anomalies in different species of turtles. * This percentage includes not only scute anomalies but also other deformities. ** These individuals are of unknown origin, most of them found in recovery centres and/or released from captivity.

TABLA 3. Anomalías en diferentes especies de tortugas. * Este porcentaje incuye no sólo anomalies escutales sino otras deformidades. ** Individuos de origen desconocido, muchos de ellos procedentes de Centros de Recuperación de Fauna y otras formas de cautividad.

Species	Locality	% anomalous individuals (N)	Reference
Chelydra serpentina	Great Lakes-St Lawrence river, Ontario (Canada)	0-21.4* (120-446) hatchlings	BISHOP *et al.*, 1991; BISHOP *et al.*, 1998
Chrysemys picta	Saskatchewan (Canada)	12.0 (50) males 34.0 (53) females 16.0 (25) juveniles	MACCULLOCH, 1981
Chrysemys picta	Thomson, Illinois (USA)	20.7 (641) hatchlings	F. Janzen (2006), personal communication
Chrysemys picta	Stevenson, Washington (USA)	58.6 (87) hatchlings	F. Janzen (2006), personal communication
Emydoidea blandingii	Nova Scotia (Canada)	6.9-15.9 (101-110) hatchlings	STANDING *et al.*, 2000
Emydura macquarii macquarii	South-East Australia	8.0-31.6 (19-232) hatchlings	JUDGE, 2001
Emys orbicularis	Genf (Switzerland)	23.8 (260) adults	MOSIMANN, 2002
Emys orbicularis	Minorca (Spain)	3.3 (30) adults	VEYSSET, 2005
Emys orbicularis	Zamora (Spain)	19.0 (78) all individuals	ALARCOS *et al.*, 2005
Emys orbicularis	Western Poland	54.0 (27) adults	NAJBAR & SZUSZKIEWICZ, 2005
Emys orbicularis	rivers Pripyat and the Western Bug (Belarus)	30.8 (65) males 9.2 (174) females 12.5 (24) juveniles	DROBENKOV, 2005
Emys orbicularis	Brandenburg (Germany)	7.7 (52) adults	SCHNEEWEISS & BECKMANN, 2005
Gopherus agassizii	Washington County, Utah (USA)	20.4	GOOD, 1984
Gopherus agassizii	Kern County, California (USA)	11.2	GOOD, 1984
Malaclemys terrapin	Southern New Jersey (USA)	22.9 (341) females 23.0 (65) males 47.7-71.0 (25-151) hatchlings	HERLANDS *et al.*, 2004
Malaclemys terrapin	Ohio (USA)	21.0 (565) hatchlings	ROOSENBURG *et al.*, 2004
Mauremys leprosa	Castroverde (Portugal)	6.5 (61) all individuals	C. Ayres *et al.*, unpublished data
Mauremys leprosa	Almograve (Portugal)	0 (16) all individuals	C. Ayres *et al.*, unpublished data
Mauremys leprosa	Ciudad Real (Spain)	6.6 (30) all individuals	C. Ayres *et al.*, unpublished data
Mauremys leprosa	Doñana (Spain)	0 (27) all individuals	C. Ayres *et al.*, unpublished data
*Mauremys leprosa***	Galicia (Spain)	21.0 (43) all individuals	C. Ayres & A. Cordero, unpublished data
Terrapene ornata	Kansas (USA)	11.0 (127) adults	KAZMAIER & ROBEL, 2001

GALLO, 1996; GONZÁLEZ RODRÍGUEZ, 1999) and the low incidence in the Doñana National Park. Nevertheless other populations with high incidence of anomalies are in apparently unpolluted areas (like Ribadavia, Almograve, Boticas or río Arnoia). Therefore an additional explanation is needed.

The effects of suboptimal conditions during incubation can be tested with experimental incubation in the laboratory (LYNN & ULLRICH, 1950). There is evidence that anomalies are more common at higher temperatures in at least two species (WOOD & HERLANDS, 1997; JUDGE, 2001; HERLANDS *et al.,* 2004), but desiccation has also been experimentally shown to increase anomalies (LYNN & ULLRICH, 1950), and both factors probably are correlated. Nevertheless, preliminary results from 31 clutches incubated under two humidity conditions do not support humidity as an important factor for the appearance of anomalies in NW Spain populations (unpublished data).

The third possibility is that a genetic mechanism is responsible for the anomalies. High proportions of anomalous turtles in Iberian populations might be due to the loss genetic diversity and inbreeding associated to the evolutionary history of this lineage, or to small population size (VELO-ANTÓN *et al*, 2008). It is interesting to note the low incidence of anomalies in Minorca (VEYSSET, 2005), where turtles have been introduced probably by Romans from non-Iberian populations (FRITZ *et al.*, 1998). Further evidence for a genetic predisposition in *E. orbicularis* to show scute anomalies is the fact that where this species is found together with *Mauremys leprosa,* the proportion of anomalies is clearly lower in *Mauremys* (0-6.6%, but 21.0% in specimens found in different localities in Galicia) than *Emys* (3-69%) (see data on Table 3 for *Mauremys* and Table 1 for *Emys*).

The significant relationship between latitude and proportion of anomalies, and the fact that anomalies are more common to the western part of the Iberian peninsula (Fig. 1), suggest that an environmental –or historical– factor is contributing to the phenomenon. There is evidence that terrestrial and semi-aquatic turtles are more subject to anomalies than aquatic turtles (ZANGERL & JOHNSON, 1957), although ZANGERL & JOHNSON's study includes not only scute anomalies but also abnormalities in sulci between scutes and therefore we do not know if accessory or lacking scutes are also more likely to occur in terrestrial turtles. In any case this fact clearly indicates that incubation temperatures and humidity are good candidates for a causal factor, but unlike our results, we would expect anomalies to increase from North to South given that Southern populations are exposed to drier and hotter climates. Genetic data of Iberian populations of *E. orbicularis* indicates a decrease of the number of alleles and genetic diversity from South to North (VELO-ANTÓN *et al*, 2008), as expected if Southern Iberia acted as a glacial refuge (FRITZ *et al.*, 1996), suggesting that lower genetic diversity is correlated with higher prevalence of anomalies.

In conclusion, our study clearly shows that scute anomalies are common in Iberian populations of *E. orbicularis*, and this suggests a genetic component for this phenomenon in the Iberian lineage. Nevertheless, the extremely high incidence of accessory scutes and other anomalies in some populations needs further study.

Acknowledgements

We are grateful to Alberto Álvarez (Madrid), Jesus Leguía and Pablo García (Salamanca), Gonzalo Alarcos (Zamora), Pedro Segurado (Portugal), Vicente Sancho and

Ignacio Lacomba (Valencia); Judit Hidalgo, Matati Pérez, Alex Portheault and Ana Andreu (Doñana) for helping us during our fieldwork. Funding was provided by Research grants from Xunta de Galicia (PGIDT01MAM-37101PR and PGIDIT05RFO37101PR) and Cátedra Filgueira Valverde - Universidade de Vigo to ACR.

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ISSN-0213-6686
Rev. Esp. Herp. 22 (2008)
Valencia

The *Revista Española de Herpetología* is the peer-reviewed scientific journal of the **Asociación Herpetológica Española** (AHE). It is indexed in/abstracted by the following services: BiologyBrowser, BIOSIS, CINDOC, Dialnet, Herpetological Contents, Revicien, and Zoological Record.