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Near-natural Incubation of *Testudo graeca soussensis* Pieh, 2000, Eggs

Moroccan tortoises are severely threatened in their native country by factors such as increasing aridity, overgrazing of their natural habitats, and fragmentation of the land, but also as a result of their being legally traded within Morocco and illegally exported to Europe. For all of these reasons, populations have been diminishing and their densities today are sparse. Older specimens of more than twenty years of age hardly exist anymore, and reproduction is on the decline (El Mouden *et al.* 2003, Znari & Mace 2003). To date, Moroccan laws have only been regulating the export of tortoise products and live animals in the context of CITES, but not their trade within Morocco, and especially not their sale in the centres of tourism. Merely perfunctory checks at customs do not help to improve this situation either (Dupre 2001, personal observation). Field researchers therefore estimate that tourists might still be illegally exporting up to 10,000 young tortoises annually (Znari & Mace 2003). Most of the animals thoughtlessly purchased as souvenirs are in a poor state of health after having suffered the appalling conditions in the North African souqs. Even if these animals are cared for adequately once they have arrived in Europe, they often die shortly thereafter - one of the reasons why these tortoises are generally thought of as difficult to maintain in captivity.

Given this background, it is good to know that some North African varieties not only thrive in captivity, but can even reproduce easily. This is demonstrated by a near-natural incubation experiment in the lowlands of the Upper Rhine, in the region of Karlsruhe, Germany.

The parents of the offspring described below have spent the summer in an outdoor enclosure for many years. More recently, a seasonally heated greenhouse (Figs. 1, 10) was set up, and they have since been left in their outdoor accommodation throughout the year. Hibernation takes place there as well, the period of which can vary substantially with the individual despite identical environmental conditions (ca. 70-120 days). Under these circumstances, which correspond to those currently thought to be optimal husbandry conditions for European tortoises, the animals present themselves as easily cared for and very fertile. While the eggs of their very first breeding season proved infertile, all of the subsequent clutches hatched at a success rate of 100 %, irrespective of the incubation methods employed (i.e. a home-made incubator with two heating mats and nightly temperature decreases, controlled by means of a "Thermotimer"; a *Jäger* breeder; or near-natural incubation in the nest pit excavated by the female). Like their parents, the resultant juveniles are kept in the hotbed /greenhouse or an outdoor pen throughout the year. Their diet consists exclusively of wild herbs, hay, hibiscus, and prickly pear cacti.

In 2006, the female produced two clutches of six and five eggs, respectively. They were laid at two different sites, one in the outdoor enclosure and one in the greenhouse, providing a basis for an interesting incubation experiment under near-natural conditions. While the first clutch was buried in the provided nest site outside in late April, the second one followed within three weeks and was deposited in the moist root area of the greenhouse vegetation. Despite the presence of drier and therefore warmer spots, the female apparently rejected these as unsuitable.

From the time of oviposition in mid-May to the time the neonates emerged in late August, the eggs of the greenhouse clutch were left untouched, i.e., they were neither dug up nor manipulated or artificially warmed in any way. Of the clutch laid in the outdoor enclosure, only two eggs were left in place, given the poor prospects of a successful incubation. As a result of an extended cold springtime in 2006, these two "outdoor eggs" indeed showed no development whatsoever. However, all five eggs left unattended in the greenhouse eventually saw baby tortoises hatching that were apparently perfectly healthy. They emerged from their nest pit after about 102 days. The first two hatchlings were seen shortly before dusk, and one of them became "stuck" overnight, with only its nose and front feet showing above the surface (Fig. 8). Two more babies left their pit in the first rays of sunlight the following day, and the fifth specimen emerged during the course of the morning. The first four hatchlings had already stretched when they dug themselves out, and their navels were almost completely closed. As a result of being covered with the gluey remains of their eggs, they were virtually coated with sand (Fig. 7) which in the wild might not only provide good camouflage, but also some protection from dehydration. The last hatchling still showed an arched carapace with the round shape of its egg. As a result, this tortoise emerged with a piece of eggshell still attached that covered its entire carapace, and that then fell off a short while later (Fig. 7). While it too had largely resorbed its yolk sac (Fig. 9), its transversal plastral fold was still distinct. It is likely that this individual's hatching was only triggered by the movements of its siblings, which had probably left their eggs a few days earlier, but had stayed in the safety of the nest pit for some time. In the wild, neonates may sometimes remain underground for as long as three weeks before they eventually appear on the surface (Diaz-Paniagua *et al.* 1997).

During the incubation in the soil of the greenhouse, artificial influences on the eggs were limited to the occasional watering of the plant amongst whose roots the eggs had been laid,

Fig. 1. A view of the interior.

and thus only the moisture level of the nest pit area was sustained. Temperature management, other than that provided by the insulation of the greenhouse, was left entirely to the climate of the Upper Rhineland, for the greenhouse was not artificially heated at any time throughout the incubation period. Thus, the temperature of the soil around the nest area varied from 20 to nearly 38 °C during this period of time, with a diurnal temperature variation of about 5-8 °C. In a nest monitored by DIAZ-PANIAGUA *et al.* (2006) in Spain, these values varied to a much greater extent, with fluctuations of more than 15 °C during the course of a day, and of about 15 to 41 °C throughout the entire incubation period. In the greenhouse, temperatures inside the nest pit did not fall below 30 °C at night during the second half of the incubation period, whereas the values never significantly exceeded 25 °C at night in the aforementioned nest in Spain.

As with all reptiles whose sex is determined by incubation temperatures, that of Spur-thighed tortoises is fixed within a certain time window during incubation, known as the thermo-sensitive phase (MROSOVSKY & PIEAU 1991). Under constant temperature conditions, the pivotal temperature for *Testudo graeca* lies at 30.5 °C (PIEAU & DORIZZI 2005). With fluctuating incubation temperatures, however, females may also be formed if temperatures drop below this point for a longer period of time. The deciding factor for the formation of females in this case is that the majority of the embryonic development must take place above the critical temperature during the thermo-sensitive phase (GEORGES *et al.* 1994). Whether females can be produced under the near-natural incubation conditions described here remains to be determined in a few years' time. Unfortunately it is very difficult to make any predictions to this effect, since the thermo-sensitive time window varies as a function of the irregular embryonic development and thus cannot be properly determined. It also does not correlate with data obtained from incubation at a constant temperature (12-15 days within the middle third; PIEAU & DORIZZI 1981).

Fig. 2. Moorish tortoise.

Fig. 3. The fifth vertebral is fused to the fourth lateral (specimen from the near-natural brood).

the early stages and/or very high temperatures later during incubation may also have slowed down the development (comp. GEORGES *et al.* 2005).

The near-naturally incubated hatchlings from the greenhouse were found to be visibly more active on their first day above the surface than the neonates previously obtained by means of artificial incubation. They in fact appeared almost "wound-up" and explored the area of the greenhouse accessible to them (ca. 1.5 m^2) for hours on end, presumably in search of a safe shelter. This noteworthy difference in activity levels could be the result of a slower development due to varying temperatures, a longer period of time spent lingering in the nest after hatching, or an extension of the activity that began when the hatchlings dug themselves up. Alternatively, it could also simply be a response to the bright natural sunlight that they were exposed to in the greenhouse. Differences with regard to hatching weight, body shape or colouration

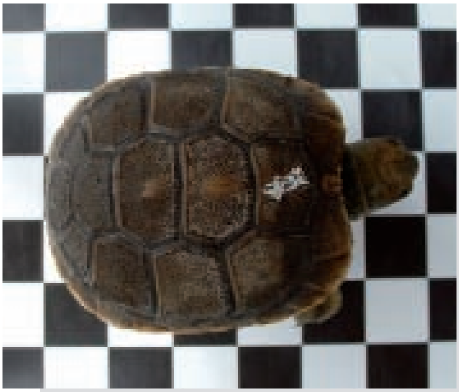

Fig. 4. Normally formed specimen (near-natural brood).

In the present experiment, the incubation period of 102 days falls within the range known from *Testudo graeca* in Spain, which may vary from 82 to 140 days (DIAZ-PANIAGUA *et al.* 2006). Earlier clutches from the same parents, which were incubated at approximately 33 °C with a six hour decrease to ca. 28 °C at night, had hatched after only 72-81 days of embryonic development, with differences of up to seven days within the same clutch. In the case of the "greenhouse hatchlings", the time span between the emergence of the first and the last hatchling was a mere 17 hours. There are several potential explanations for the almost 30% increase in incubation time relative to the eggs placed in an incubator. Next to an unknown length of time the hatchlings may have spent lingering in their nest, it is possible that the low night temperatures during

Fig. 5. Six vertebrals and five laterals each (hatched in an incubator).

Fig. 6. A baby tortoise emerges from its nest pit.

were not obvious. The average weight of these neonates obtained by means of near-natural incubation conditions at hatching was 11.9 g and thus marginally higher than that of previous clutches from the same parental pair (11.25 and 11.75 g, respectively), but the overall sample size is far too small to make a representative statement in this regard.

The same applies to scute anomalies. One of the "greenhouse tortoises" - the fifth neonate with the slowest development - emerged with an anomaly even though the maximum temperatures inside the nest were far below the maximum values recorded from nests in the Spanish wild, where hatching was still

Fig. 7. The last hatchling of the clutch, still round and with a piece of eggshell attached.

successful despite temperature peaks of up to 47.4 °C (DIAZ-PANIAGUA *et al.* 2006). In contrast to scute anomalies observed in specimens that hatched in an incubator, however, this baby showed a reduced number of scutes (comp. Fig. 3 to Figs. 4, 5).

In the future, the neonates that have experienced a slow natural development during incubation are to be reared under near-natural conditions as well. Depending on local variety and availability of food, free-ranging *Testudo graeca* tend to increase their shell length by a factor of 1.6 to 2.3 and their body weight by

Fig. 8. Starting life with a protective coating of egg fluid and sand.

a factor of 4 to 7 within the first three years of life (ZNARI *et al.* 2005). The weight of the greenhouse hatchlings will be compared to these field data and, if necessary, they will be provided with food in a restricted manner, since their older siblings grew much faster than wild specimens when provided with unrestricted access to food. ,.

In conclusion, the present study suggests that, whereas eggs of the Moroccan tortoise laid in German outdoor enclosures only have an small chance of developing, eggs laid in a greenhouse can apparently develop without human intervention and produce healthy hatchlings. However, the sample sizes of comparable broods are currently too small to make a generalized statement to this effect. In addition, the subsequent juvenile growth cannot be compared directly to that of previously obtained specimens, since the groups were raised in a different manner. With regard to the temperature-dependent sex determination, it should be noted that the incubation temperature may still have been within the “male range”. Despite air temperatures partly above those commonly experienced in the tortoises’ native country, the lower intensity of sunlight in Germany, in conjunction with the absorption and reflection at the glass of the greenhouse, would be expected to warm up the soil to a lesser extent than in the Mediterranean. Until it is known whether the described incubation conditions can also produce female offspring, it is recommended that keepers limit this unassisted, near-natural type of incubation to “accidents”, i.e., to clutches that have been overlooked. Female specimens should be produced in the controlled environment of an artificial incubator. However, the ease of near-natural incubation in outdoor enclosures described above shows that it might be advisable to watch out for newly hatched babies during summer and to take precautions to protect these from accidents and predators.

Fig. 9. Even though the yolk sac has been resorbed, the transversal plastral fold is still evident.

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Fig. 10. Outdoor accommodation.