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# New project to save the Albera tortoise from extinction

The native tortoise to Catalonia is a local form of the Western Mediterranean tortoise, *Testudo hermanni hermanni*. These tortoises are found in the Eastern Albera mountain range at the foothills of the Pyrenees close to the French border. For the purpose of this publication these tortoises will be referred to as Albera tortoises. Situated in north eastern Catalonia just outside the town of Garriguella, where vineyards and olive groves blend into the beautiful Albera mountain landscape sits the Centre de Reproduccio de Tortugues de l'Albera (CRT). This is the coordinating facility for

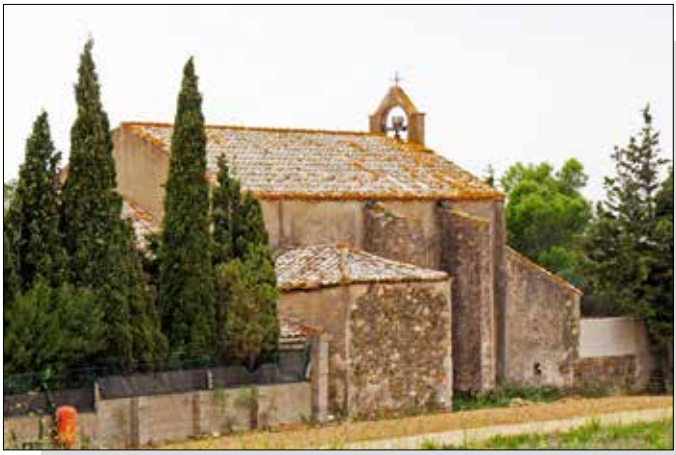

Fig. 1.
CRT building.
Photo: B. Pfau

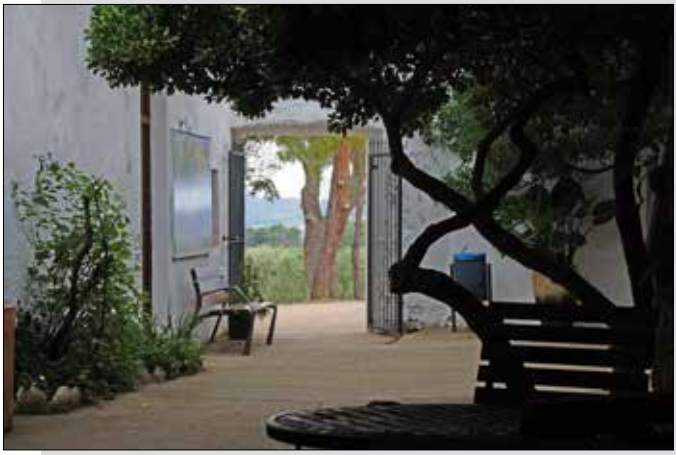

Fig. 2.
CRT entrance.
Photo: B. Pfau

the protective measures implemented by law to conserve these emblematic native tortoises (Fig. 1, 2). At the CRT a dedicated team works to reinforce the wild population numbers by successfully hatching 200 to 300 pure Albera tortoises per annum which are later released into their natural range (Capalleras *et al*. 2013). To provide this number of hatchlings annually there is a large and carefully regulated breeding group of pure adult Albera tortoises which are in permanent residence at the CRT (Figs. 3, 4a, 4b). Since 2013 there is even an extension of land behind the main tortoise enclosures, where the new outdoor enclosures for raising the hatchlings could be constructed (Fig. 5), and sometimes even wild Albera tortoises enter this area to lay their eggs or to feed on the wild herbs (Fig. 6). This ideal location and the close proximity to the wild range of the Albera tortoises not only provides for an ideal and natural environment for these resident tortoises, but also makes the Centre de Reproduccio de Tortugues de l'Albera a vital resource in the struggle to conserve this critically endangered tortoise.

**The decline of the Albera tortoise**

More than 3000 captive born Albera tortoises hatched at the CRT have been released into the wild at carefully selected release sites over the past 30 years. This will continue into the future (Capalleras *et al.* 2011). The CRT has implemented several long term studies of the population density (Fèlix 1999, Budó 2001, Budó & Mascort 2017). The Albera tortoise habitats have often been affected by forest fires, and studies will investigate the expected recovery of the populations in the naturally regenerated habitats (Santos *et al.* 2016). The CRT surveys have been done always with the same method, and preferably by the same persons, but the population decline, even in good tortoise habitats, is evident. The aspiration is to reinforce the population numbers of the Albera tortoise in its local range. Despite this effort the wild population numbers continue to decline rapidly. Previous tortoise protection projects and habitat enhancements in the Albera range have not had the desired effects to fully protect these tortoises (Vilardell-Bartino *et al*. 2015). It is therefore high time to put additional plans in place to preserve this precious Catalan tortoise for future generations to appreciate as we do today. On the 5th of December 2016 the CRT hosted a specialist meeting attended by Albert Bertolero, Joan Budó, Enric Capalleras, Xavier Capalleras, Marc Cheylan, Beate Pfau and Sergi Vila de Vicente. Jenar Félix and Saliha Beddek (Zenboudji)

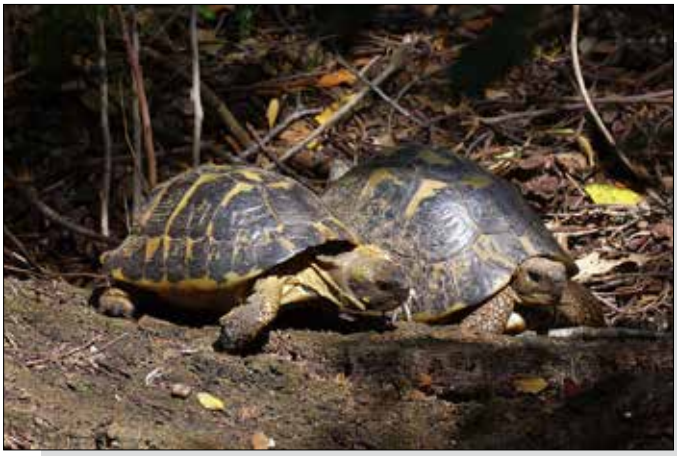

Fig. 3. Courting tortoises in the CRT. Photo: H.K. Pfau

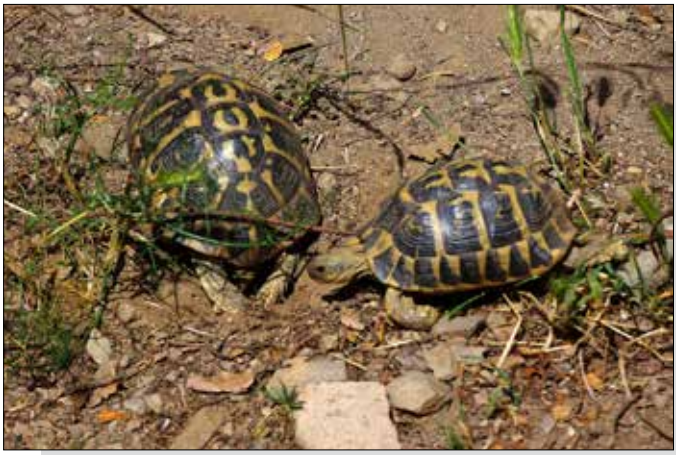

Fig. 4a.
Nesting tortoise in the CRT.
Photo: H.K. Pfau

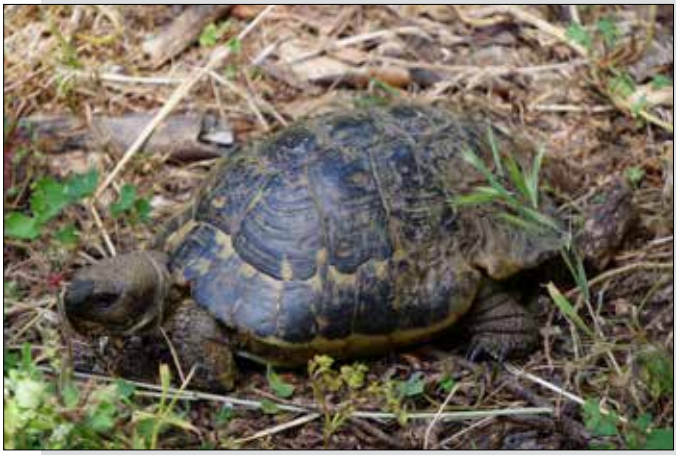

Fig. 4b.
Old and scarred Albera tortoise from the breeding group in the CRT.
Photo: H.K. Pfau

could not come to Garriguella, but they are members of the working group, too. The purpose of this meeting was to discuss the possible reasons for the worrying decline of the Albera tortoise population and as a result a research and conservation project was put in place. This project will be conducted in close cooperation with Albert Martínez-Silvestre and Joaquim Soler from the Centre de Recuperacio d'Amfibis i Reptils de Catalunya (CRARC), and under the coordination of Joan Pretus (Universitat de Barcelona). Below we will describe the actual status of this project and we invite discussion with the reader.

**Sensitivity of the Albera tortoise**

Research by a group of ecologists on a comparison of the genetic structures of Western Mediterranean tortoises (Zenboudji *et al*. 2016) concluded that the Albera tortoise has a lower genetic diversity compared to other local forms of Mediterranean tortoises. The recalculation of the (genetically) effective population size has given a value of only about 80 animals which would be classified as "high extinction risk from inbreeding" for avian and mammalian populations (Reed *et al*. 2006). Inbreeding effects in reptiles have been demonstrated in very small, isolated

populations of the European adder (*Vipera berus)* in southern Sweden (Madsen *et al*. 1996) and an introduced population of the Wall lizard (*Podarcis muralis)* in England (Michaelides *et al*. 2016), but not yet in chelonians. On the other hand there are tortoise populations with even smaller effective population sizes which do not show any signs of negative effects of inbreeding on their overall vitality, see for example Milinkovich *et al*. (2013) on their research with the Galapagos tortoises.

The Albera tortoise reproduces less prolifically and in general terms seems more fragile and less vigorous compared to closely related forms of *Testudo hermanni hermanni* (namely the local forms from north-western Menorca and from southern France). It has been observed that female Albera tortoises typically lay fewer eggs than females from other localities despite both parents being of similar size and weight. Also, the hatching rate is lower in Albera tortoises, and a high number of advanced embryos or newly hatched tortoises do not survive, the reasons for this are unknown but are being explored in this project. Interestingly, the malformation rate is however not elevated.

Fig. 5.
CRT new outdoor enclosures for headstarting hatchling Albera tortoises.
Photo: B. Pfau

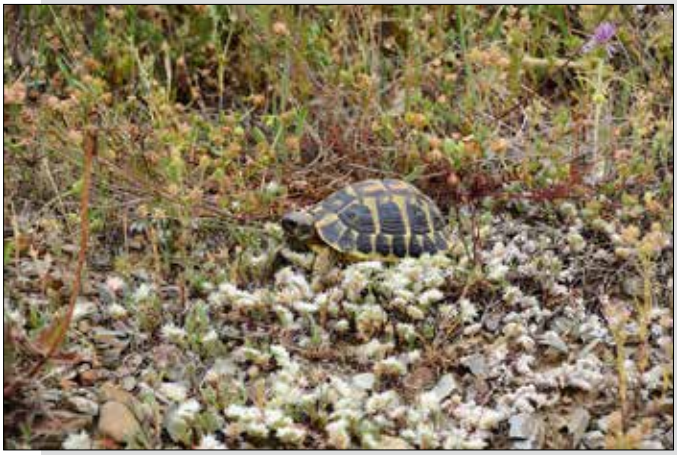

Fig. 6.
Young wild female Albera tortoise which has come to the CRT extension area for feeding.
Photo: H.K. Pfau

Hatchlings of the Albera tortoise will typically weigh half the weight of their close cousins from other localities. In captivity under near identical conditions it has been noted both in private collections and at the CRT that the Albera hatchling will grow at a much slower rate than other hatchlings of *Testudo hermanni*. The Albera tortoise of any age is highly sensitive to stress and seems particularly prone to a number of infections. To find out whether the reduced vitality of the Albera tortoise is due to inbreeding, protozoan infestations, virus infections, environmental toxic influences or other unidentified infections, a thorough veterinary examination and screening will be conducted as the first stage of the conservation project. When assessing the health status of an individual Albera tortoise from blood parameters they must be compared to reference values. For the western Mediterranean tortoise reference value ranges have already been established (Andreani *et al*. 2014, Fiorucci *et al*. 2013, Macrelli *et al.* 2013). All tortoises which are to be examined and screened should live under nearly identical conditions. If the values from captive and wild Albera tortoises are within normal ranges for clinically healthy tortoises, a more detailed examination might be necessary to find out whether they have a subclinical disease or a deficit of certain minerals or vitamins. In two populations of *Caretta caretta* it could be shown that even subtle deficits could have a negative influence on hatching success or embryonic mortality (Trocini 2013) and we want to know about this in the Albera tortoise, too.

**Enhancing the genetic diversity of the Albera tortoises**

It is imperative that we establish whether a genetic enrichment (introducing genes from tortoises of the closest local form) will, in fact, boost the vitality and robustness of the Albera tortoises while preserving as much as possible the unique traits and special adaptations associated with it (keep locally adapted alleles, see Weeks *et al*. 2011).

**Significance of the genetic diversity of a population**

There are many scientific and amateur publications on the significance of genetic diversity for the overall health of species or populations, but there is no applicable statistical guide or method of comparing more or less healthy populations or to measure the deviation from a defined target value for genetic diversity. Apart from this the traits used for population genetic research (mitochondrial or nuclear DNA or other metered values) do not have any ecological significance. There are also many publications suggesting the presumed impact of inbreeding depression or, conversely, outbreeding depression on the overall health status of populations, but again there is no scientifically sound guide or method for measuring this. Additionally, the majority of these publications do not apply to populations of reptiles surviving in their natural environment, but there are some recommendations from quantitative genetic theory calculations (Frankham *et al*. 2014). Nevertheless it could be shown for many kinds of animals (vertebrates and invertebrates) that reduced genetic diversity probably contributes to many components of phenotype and fitness, including metabolic efficiency, growth rate, reproductive physiology and disease resistance (Alacs *et al*. 2007, Keller & Waller 2002).

**Former distribution of Testudo hermanni hermanni in south-western Europe**

A survey of ice age tortoise fossils in France has revealed that tortoise populations were abundant in southern France and continued through an area of continuous distribution to the Pyrenees (Cheylan 1981). On the Spanish side of the border the populations of *Testudo hermanni* stretched from the Pyrenees southward to the Malaga region and westward into Portugal (Morales Pérez & Sanchis Serra 2009). At this time a continuous population existed from France to the Iberian Peninsula (Bertolero *et al*. 2011). The population then split geographically and resulted in separate

French and Iberian localities. Unfortunately the last of the French tortoises near the Pyrenees disappeared in the 1960s (Cheylan 2001). The Iberian Peninsula populations were also greatly reduced, partly to human influence and habitat loss, until only the Albera population remained (Fèlix *et al.,* 2006). Genetic research has proven that about 19,000 years ago there must have been a significant population reduction of the Spanish tortoise population resulting in a "genetic bottleneck". This coincides approximately with the last glacial maximum (Zenboudji *et al*. 2016). In historic times, perhaps as recently as 100-450 years ago, there was another significant population decline of the Albera tortoises and this can be directly attributed to increased agriculture resulting in the habitat loss and destruction in the Albera range (Capalleras et al. 2013).

*Testudo hermanni* populations on the Balearic Islands were introduced by sailors around 3000 years ago, presumably as a fresh food source (Mayol 1985, Pinya & Carretero 2011, Fritz *et al.* 2006). The tortoises introduced to Mallorca and north-western Menorca most likely originated from the northern Catalonia region. *Testudo hermanni* populations which exist on the Iberian Peninsula today are a result of reintroductions originating mainly from Menorca and perhaps also Mallorca (Soler-Massana *et al*. 2002a, Soler-Massana *et al*. 2002b, Bertolero *et al*. 2007, Mateo 2011, Drechsler *et al*. 2016).

**Finding the local form of *Testudo hermanni hermanni* which is the closest relative to the Albera tortoise**

Abbreviations: A: Albera, M: Mallorca or Menorca

It is very important to preserve the Albera tortoise with as many of its unique traits and adaptations as possible (Fig. 7). When introducing foreign genes, it is imperative to identify the genetically closest local form of *Testudo hermanni hermanni* for this purpose, as recommended for any genetic rescue project (Frankham *et al*. 2011). In Zenboudji *et al.* (2016) the *Testudo hermanni hermanni* from north-western Menorca and from southern France have been shown to be the nearest relatives to the Albera tortoises, but the Albera tortoise is genetically differentiated from any other local form which had been included in this publication and should therefore be treated as a separate genetic management unit. In other tortoise species, like the Desert tortoise, *Gopherus agassizii* (Averill-Murray & Hagerty 2014) or the Geometric tortoise,

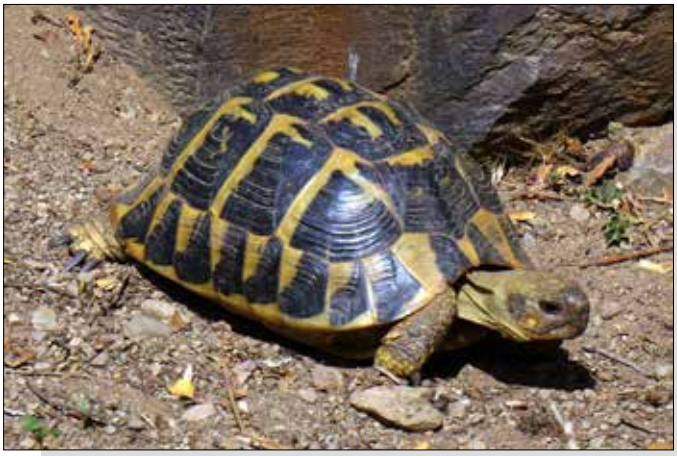

Fig. 7.
Albera tortoise from the breeding group in the CRT.
Photo: H.K. Pfau

*Psammobates geometricus* (Greig 1979), it has been shown that care must be taken not to introduce tortoises from excessively diverse populations and thus enhance the risk of outbreeding depression. Interestingly, the local form of *Testudo hermanni hermanni* from the Balearic island of Mallorca (Fig. 8) is very similar to the Albera tortoise in external appearance (form/shape, size and coloration). However, there has not yet been genetic comparative analysis for evaluation done on these tortoises using the same method that was applied to the Menorcan and southern French tortoises. The University of Montpellier already has a stock of blood samples from geographically different Mallorcan populations which could be used for this purpose. Over the years there have been many seizures and confiscations of *Testudo hermanni hermanni* from Menorca. Many of these animals still survive in captivity today and could easily be identified and used for analysis and experiments relating to this project. It will be necessary to establish if these are in fact the most genetically suited tortoises for the genetic introgression experiments. Additionally, because there are two genetically different *Testudo hermanni hermanni* populations surviving on Menorca (Massana *et al*. 2013, see also Fig. 9 and Fig. 10), each tortoise proposed for introgression would have to be individually analyzed genetically to assure it has originated from the desired north-western population. Firstly the results from the genetic evaluation of the Mallorca tortoise samples will have to be considered to determine if these tortoises are a better genetic option for the introgression experiment.

Ecological comparison of the Albera tortoises with other local forms with greater genetic diversity, living in similar habitats

In the department Var in southern France there are several populations of *Testudo hermanni hermanni* with some genetic influence of Corsican tortoises, or even of *Testudo hermanni boettgeri*, which had been released there (Guyot & Pritchard 1999, Cheylan *pers. comm.*). The population density is between 2 and 10 tortoises per hectare. These tortoise populations are threatened by habitat loss or unsuitable habitat creation due to reforestation projects in this region. Nevertheless, even with many predators, predominantly wild boar, the tortoise populations are increasing. Another population of tortoises which could be used for ecological comparison survives in the Bosco de Mesola in a rather dense forest in the Po delta, Italy (Mazzotti 2004). This

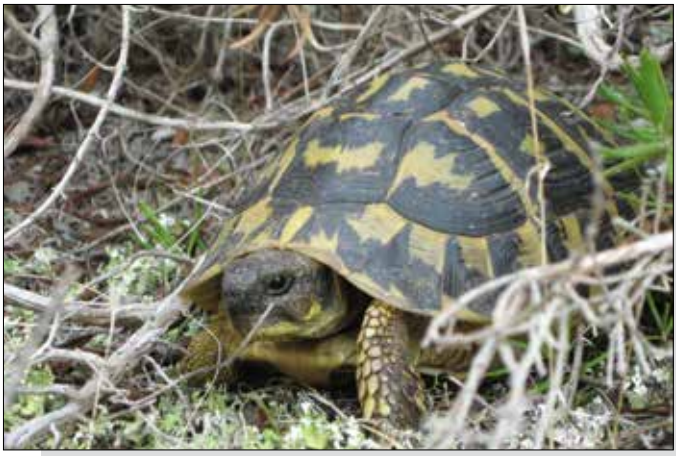

Fig. 8.
A Mallorca tortoise.
Photo: O. Kuss

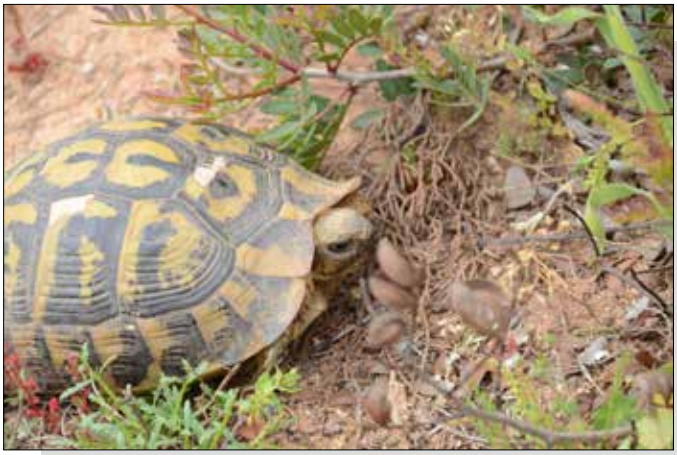

Fig. 9.
Tortoise of the north-western local form of Menorca.
Photo: B. Pfau

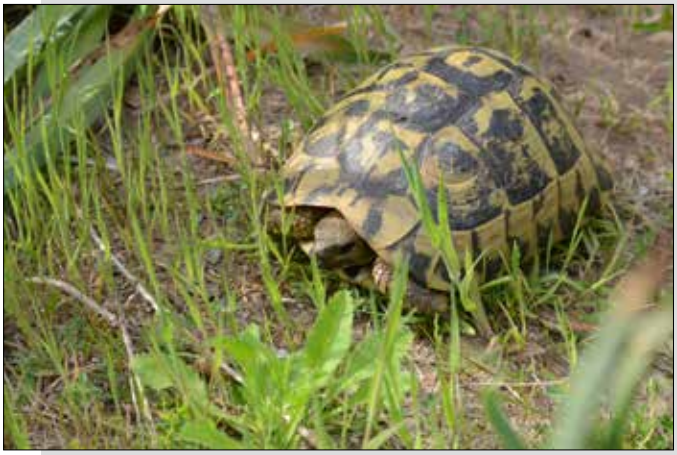

Fig. 10.
Tortoise of the south-eastern local form of Menorca.
Photo: B. Pfau

is a population of hybrids comprising of *Testudo hermanni hermanni* and *Testudo hermanni boettgeri*. It should be noted that this area is the presumed natural border of these two subspecies and therefore it is not known whether this hybridization is a natural occurrence or whether some released tortoises have had influence.

A third population which could be included for ecological comparison survives in the Delta d'Ebre in the province of Tarragona in north-eastern Spain (Bertolero 2013). These tortoises originate from Menorca and were relocated to this region by man. The geographical location of the Delta d'Ebre near the coast closely reflects the habitats to be evaluated in the natural park of Cap de Creus for the introgression project.

**Experiments on enhancing the genetic diversity of the Albera tortoise**

The first breeding experiments between the Albera tortoises and the preselected genetically appropriate tortoises will take place in the Reserva Natural Remolar-Filipines. The breeding site selected is a large fenced in, predator proof area. The

location is sufficiently distant from the natural populations of Albera tortoise to avoid any accidental contamination and unsupervised contact with wild tortoises or even privately owned tortoises.

Some notes worth considering for the reproduction phase of the project are as follows.

We predict that the "A" males will be at a disadvantage reproductively compared to the "M" males chosen for the breeding experiment due to the already recorded vitality of both forms.

Females of "M" tortoises may retain sperm from "M" males from previous pre-experiment mating, although being housed and mated by "A" males.

After the breeding phase of the project concludes it is very possible that "A" females will continue to produce hybrids due to retained sperm from "M" males.

Therefore, it will be necessary to identify with certainty whether a hatchling produced during this phase of the project is the desired result of experimental cross breeding between "A" and "M" tortoises or a result of retained sperm in the applicable female. It will be interesting to see if these hatchlings are recognizable by external appearance only.

Once the most applicable form of *Testudo hermanni hermanni* ("M" tortoise) has been identified for the introgression period with the Albera tortoise, we must then organize the acquirement of these animals. If the form from Menorca is found to be the most suitable, the best suited animals will be selected from confiscated groups already in captivity. If the form from Mallorca is selected the necessary paperwork will need to be completed to receive animals from there. The acquirement of "A" tortoises cannot originate from the existing colony in residence at the CRT because this would disrupt the carefully managed dynamics and social structure of this long established group. There are, however, a trio of Albera tortoises in residence in the CRARC and there are some pure Albera tortoises in private collections. Both of these sources when identified may possibly be given to the Albera tortoise project for the purpose of breeding under special contract.

For our experiments we will use trios (one male with two females) which will be kept together year-round, in large enclosures and under very similar conditions during the experiment. Each combination will be represented by two trios.

This means that we shall have to assemble:

Two trios of one male "A"with two females "A"

Two trios of one male "M" with two females "A"

Two trios of one male "A" with two females "M"

Two trios of one male "M" with two females "M"

As soon as we have the first hatchlings we could think about measuring the fitness and the vitality of these animals.

Trial of the tortoises from the breeding experiments in natural habitats-

Reintroduction vs. genetic reinforcement of the existing population?

There are many established populations of *Testudo hermanni hermanni* in Catalonia, which now live inside the indigenious range of the species, in areas where the natural populations have disappeared (Bertolero 2013). These populations have been reintroduced, more or less on purpose, and these tortoises are not native in the narrow sense of the term as detailed in the reintroduction guidelines of the IUCN (IUCN/SSC 2013). As far as we know these reintroduced tortoise populations in Catalonia are all descendants of tortoises from the Balearic islands. The tortoises perform the same ecological function in their habitats as the native tortoises have done before their disappearance. In the Albera the situation is different: The indigenious population still exists, but is in decline. A population reinforcement with captive bred pure Albera tortoises was not effective, the population size could not be stabilized. Therefore the aim of our project is to find out whether

a genetic enrichment by introduction of a certain, probably low, percentage of genes from another local form will help to stabilize the population of the Albera tortoise in its habitat.

It will never be an option to introduce pure tortoises of Balearic origin in the Albera range itself, and thus create just another reintroduction project with non-indigenious tortoises here!

**Habitat requirements of the Albera tortoise**

The Albera tortoise (the Catalan *Testudo hermanni hermanni*) is typically darker than other forms of this subspecies. There are various suggested reasons for this which we will now discuss. Compared to other native habitats of *Testudo hermanni hermanni*, e.g. Corsica, Menorca, Sardinia, the Albera region hosts higher humidity and is typically a more shaded habitat so perhaps this is a contributing factor for this dark adaptation. Increased camouflage in these shaded habitats along with the notably dark soil of the Albera Mountains may also be considered along with the theory of thermoregulation advantage due to this dark coloration. In fact, the reason behind the dark coloration of the Albera tortoise is not known for certain, but interestingly on Mallorca a population of *Testudo hermanni hermanni* survives with some specimens becoming very dark with age (Soler-Massana *et al.* 2001). Samuel Pinya (from the University of the Balearic Islands) has informed the Albera tortoise project team that there is currently a study being conducted by students on the physiology and ecology of these tortoises. This research might provide an explanation of the Albera tortoise's dark coloration. In historic and prehistoric times Mediterranean tortoises mostly survived in a landscape which was dominated by holm oak (*Quercus ilex*) and to a lesser extent downy oak (*Quercus pubescens*). Today, the natural tortoise habitats throughout the Mediterranean region have dramatically changed. Different tree species now populate the landscape and have

Fig. 10.

therefore altered the natural light intensity and shade patterns reaching the ground level the tortoises occupy. Dramatic changes to the natural habitats of Mediterranean tortoise species can also be attributed to man. Bushfires have an immediate and dramatic effect on natural habitats of tortoises with frequent bushfires in some regions. These unfortunate events have a devastating impact not only on the effected habitat and local ecology but dramatically and rapidly reduce the native tortoise population. In the case of the Albera tortoise there is no recovery of normal population density after these events. This unfortunately has proved a tragic and massive contributing factor to an already low wild population. The result of introducing captive bred/head-started tortoises from the CRT has done little to improve this situation due to the ferocious nature and lasting impact of bushfires. Other contributing factors caused by humans that are negatively impacting the Albera tortoise population are intensive viticulture resulting in frequent plowing of the soil and sometimes include the use of pesticides, reforestation with non-native tree species which are casting greater shadow than is historically natural and also scrub encroachment because of discontinued extensive livestock grazing (Vilardell *et al*. 2012). It has been suggested that the northern slopes of the Pyrenees in France, consisting of open forests of cork oak (*Quercus suber*), could offer very suitable habitats for the Albera tortoise reintroduction plan. Historically tortoise populations in this area had been very successful (Cheylan 1981), but these tortoises are now completely extinct in the wild. Unfortunately, in this region there are no government protected areas or parks which could be used for reintroductions and it would prove too complicated to get a reintroduction permit for a protected species in France.

**Evaluation of possible tortoise habitats in the Natural Park Cap de Creus**

Historically and pre-historically tortoises occupied the region that incorporates the Cap de Creus natural park. This area has been protected by LLEI 4/1998 because of its biological richness, which comprises Mediterranean and continental elements, its extraordinary geology and its beautiful landscape. There are three specially protected areas, among them the Serra de Rodes with the information center in the old monastery.

In 2000 there was an attempt to reintroduce the Albera tortoise in the Cap de Creus region, but this project was unsuccessful. The reasons have been identified as:

Release areas being too close to roads, so that car run-overs, illegal collection as pets, and dog bites led to a high mortality or loss rate of the released animals

Dense predator populations (martens, foxes and badgers, and especially wild boar) and no alternative prey species for them, so that these animals specialized on preying on tortoises

Lack of connectivity between the habitats due to terracing of the area without passages for tortoises and other slow-moving animals

Tortoise reintroductions can only be effective if the animals are not released into poor quality or otherwise inadequate habitat, as shown for Desert tortoises, *Gopherus agassizii* (Nafus *et al*. 2017). The reintroduction of *Testudo hermanni hermanni* in the Cap de Creus region (northeast of Spain) is a very important phase in the Albera tortoise population recovery project. We will evaluate how the "genetically enriched" tortoises adjust and thrive in the natural habitats. Suitable new introduction "zones" will therefore need to be identified. These areas on the Cap de Creus are separated from the modern Albera tortoise range, which will prevent uncontrolled mixing of the tortoises from the experiments with the wild Albera tortoises. It will be necessary to conduct a complete evaluation of the Cap de Creus region to assess the best suited areas for the reintroduction of the tortoise. We shall perform a statistical analysis using "Maxent" software. Analysis from the Albera Mountains (where *Testudo hermanni hermanni* still survives wild, Villero *et*

*al.* 2017) will be used in comparison to the data retrieved from the analysis in the Cap de Creus region to identify potential reintroduction areas. Many variables will be employed to determine this including, annual rain fall, temperature, radiation index, humidity levels, wildfire risk, plant typology and land use, among others. The evaluation will run in conjunction with evaluating the habitats of the Spanish Pond turtle (*Mauremys leprosa*, Fig. 11).). This turtle species currently survives with good populations densities in the Cap de Creus region and the year-round access to fresh water for the reintroduced tortoises may prove to be very important due to the dry and windy climate of this region. When these data are collected, they will be entered into the Maxent software and in turn will produce an accurate statistical analysis depicting the distribution zones for the reintroduction of tortoises in the Cap de Creus area. The results will be depicted on a map of the area, with a range of colours marking the areas depending on the potential suitability. This information, along with previous information based on the knowledge of Albera tortoise experts will be accumulated to allow the Albera tortoise project team to choose the most suitable reintroduction sites. Although some sites have been previously suggested, especially in the Serra de Rodes (Ferrer & Filella 2011), it is important that all proposed sites will be scrutinised to determine those most suitable as future tortoise habitats. Primarily used for extensive livestock grazing, the natural vegetation and overall topography of these somewhat coastal areas were inhabited by tortoises until about 1970 and would perhaps still make a hospitable environment for tortoise introductions. (Fig. 12a, b).

Aspects like the abundance of predators, human disturbance, proximity to human settlements and roads and the local plant typology will all be carefully considered when determining the best release sites. The above efforts to determine the most suitable areas for the reintroduction of tortoises into the Cap de Creus region is a very important phase of the overall Albera tortoise project, with the main objective being the long term survival of this unique tortoise species. There are now sponsors for a new tortoise reintroduction project in the Natural park of Cap de Creus. Locating and evaluating suitable natural tortoise habitats, and measures for habitat improvement to avoid or remedy the negative impacts listed above, will prove to be one of the critical success factors in this phase of the project.

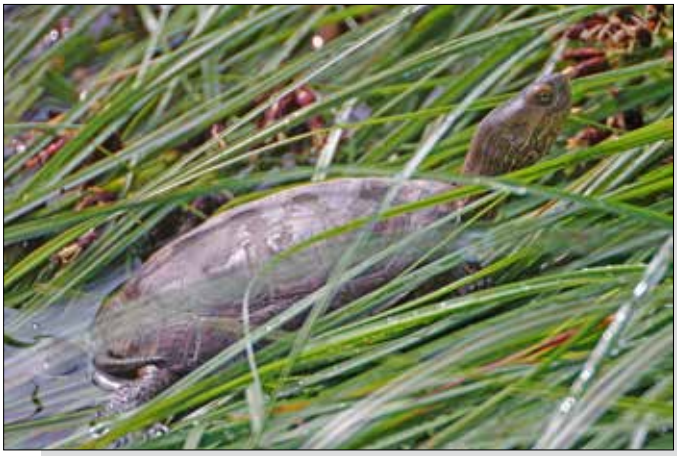

Fig. 11.
*Mauremys leprosa*.
Photo: H.K. Pfau

**Decision which experimental animals we will use for the genetic reinforcement of the Albera tortoise**

At this phase of the project sufficient data will be acquired from the results of the breeding phase to make an informed choice on which tortoises will be most ideal for release and in which location in accordance with the reintroduction guidelines of the IUCN (IUCN/SSC 2013). These results may decide to use only one combination of the aforementioned "A" and "M" hybrids, or perhaps both. From these results it will then be decided whether to release exclusively hybrid "A"x"M" tortoise groups or whether it would be more beneficial to build up mixed groups consisting of hybrids but also reinforced with pure "A" tortoises of a similar age and size from captive breeding at the Centre de Reproducció de Tortugues de l'Albera (CRT).

**Release method**

The most successful method of tortoise reintroduction projects have resulted when adult tortoises are released (Bertolero *et al*. 2007). This is because with sexually mature tortoises in place it is not necessary

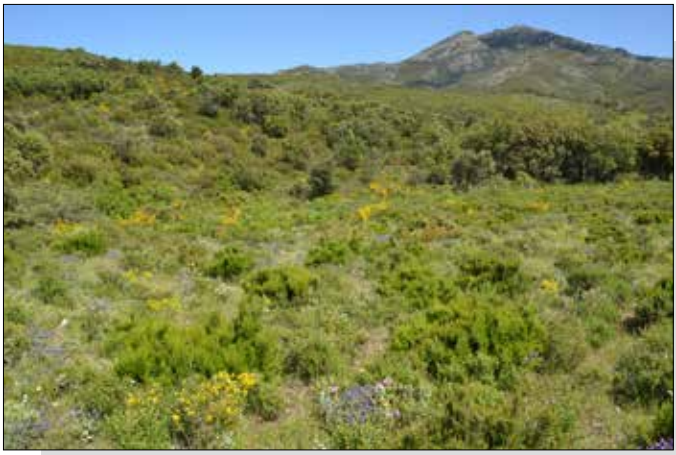

Fig. 12a. Possible tortoise habitat in the Cap de Creus. Photo: B. Pfau

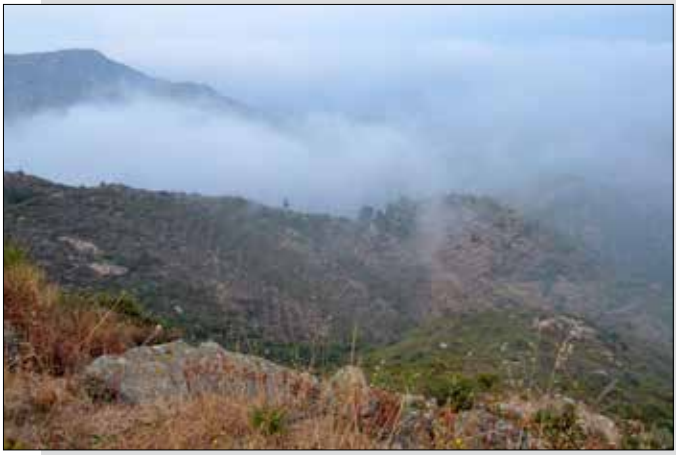

Fig. 12b. Overview of the Serra de Rodes area on the Cap de Creus. Photo: B. Pfau

Fig. 13.
Juvenile from the CRT extension area of the CRT having attained the size for release in an area with high juvenile tortoise predation.
Photo: B. Pfau

to repeat introductions annually for an extended time. These tortoises will start reproducing almost immediately and therefore gradually build up an adapted population with the optimal density for that special area. By releasing such adult specimens the goal is not necessarily for them to survive for a long time, but that they produce many offspring naturally and without any human intervention. It is important that the release sites for these projects must be as predator free as possible. When using this approach of releasing adult tortoises, the location and method must be carefully considered, factoring in the possiblity of future migration of naturally occurring populations. In the Delta d'Ebre tortoise project, the adult specimens which were released many years previous remained in the original release area, however it was observed that subsequent younger specimens, which had hatched naturally from the eggs of the released adults, migrated to another area and are establishing a growing population there. For the Albera tortoise project it is not possible to use this approach of releasing adult specimens because there are not enough pure adult "A" tortoises available which are fit and suitable for release into a new habitat. The risk of losing the precious Albera adults is far too great. It is possible to release young tortoises which have been kept in captivity for a predetermined time to make them less vulnerable to predation. This is a practice called "head starting" because the tortoises are grown to a size which makes them more robust and less prone to be attacked by the numerous predators in the Cap de Creus region. The CRT uses this method in the Albera habitats because of the high density of predators for small tortoises (Fig. 13). Using head-started juveniles has proved successful for the release of aquatic turtle species but has proved an inferior method in tortoise species. This is because the head-started tortoises grow accustomed to captive life and lose their natural fear and protective instincts. They might cease to recognize the threat of predators due to their fearless familiarity with humans, a behavior learnt from their years in captivity. They may not retreat their limbs or head into the protective bony shell as their wild counterparts would, leaving them highly exposed to attack, mutilation andmaybe leading to death. Hatchling turtles or tortoises are very prone to predation when they move around a lot in the open. In previous tortoise release projects hatchlings have been mostly released in spring, coinciding with an abundance of food in these new environments. But it was observed that the

hatchlings appeared unsettled in their new environment and began to wander in the open, possibly seeking familiar environments. This resulted in the captive bred hatchling tortoises exposing themselves far more than a wild hatchling would. For the Albera tortoise project, we suggest the best method for releasing tortoises is directly after hatching. The autumn climate will encourage the tortoises to decrease their appetite in anticipation for hibernation. This will result in the tortoises being less active and to primarily seek cover. When they emerge from hibernation in spring their surroundings will be familiar and promote natural behavior, including defensive responses to predators. This "hard release" method of introducing hatchlings immediately ensures no pure adult Albera tortoises are lost in the natural areas of the Cap de Creus. As was previously mentioned these tortoises are too few and precious to risk. As a result, a lot of hatchling tortoises will have to be hatched via the breeding project phase for quite a few years and released immediately. Being of a secretive nature at such a young age, it will not be possible to observe these specimens at regular intervals in their adopted natural environment. It will take some time for these tortoises to mature to a conspicuous size and then they can be counted, and the project as a whole can be evaluated.

**Check for long-term effects**

It is possible that the first generation of the hybrid tortoises, hatched during the breeding phase of the project, will show an enhanced vitality compared to original forms of pure "A" tortoises. (This enhancement is called hybrid vigor or heterosis).

The success of genetic rescue measures of populations with high extinction risk from inbreeding has been evaluated theoretically (Frankham 2015, Frankham 2016), but it has not yet been proven in a natural habitat with tortoises. On the other hand, it is also possible that the second, or even the first, generation of "hybrid" animals might show reduced fitness when compared to the pure (parental) local forms ( = outbreeding depression). Therefore the effects of a genetic rescue project such as ours should be tested for at least two generations, if possible, in a natural habitat (Edmands 2007). Of course the reintroduced tortoises will have to undergo also a vet check when they are handled, like any other tortoises that are released into a natural habitat (Martínez-Silvestre *et al*. 2013). When the selected tortoises previously mentioned are released into the selected release sites in the Cap de Creus, and have naturally reproduced, we can measure the success of the experiment to enhance the genetic diversity of these naturally occurring tortoises.

**Summary**

The main objective of the Albera tortoise project is to create and build up a natural population of healthy tortoises which remain as close as is possible to the Albera tortoise (Fig. 14).

This population should be stable and self-sustaining without human intervention and the population density should remain at 3 – 10 tortoises per hectare.

In the future it can then be decided whether tortoises which have inherited additional "M" genes should be released to reinforce the surviving populations within the Albera range, thus conserving a tortoise form as close as possible to the "true" Albera tortoise, while cautiously enhancing the genetic diversity of these populations.

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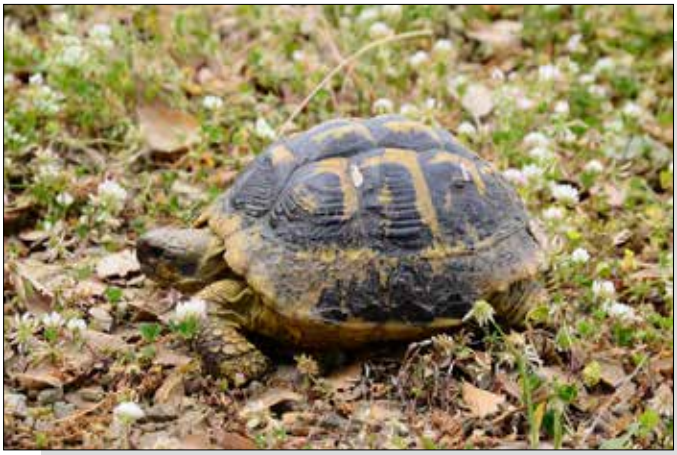

Fig. 14.
Old "wild" tortoise female from a natural habitat near Garriguella. Photo: B. Pfau

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