Invasive pathogens threaten species recovery programs

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Captive breeding and re-introduction is integral to the recovery of many threatened species [1], but such practices carry an associated risk of introducing exotic and potentially unknown pathogens into naïve settings. Amphibians are facing a mass extinction crisis and an emerging pathogen, *Batrachochytrium dendrobatidis*, described only in 1998, is now recognised as a principal driver of these declines [2]. Debate rages about the role of invasion [2,3] versus climate change [4] in determining the distribution of *B. dendrobatidis* and chytridiomycosis. The severity of the threat from anthropogenic spread is recognized by the recent decision to list chytridiomycosis as a notifiable disease by the OIE (World Organisation for Animal Health) [5]. Case-studies documenting anthropogenic spread are rare, however. Here, we report that native island populations of the IUCN red-listed Mallorcan Midwife Toad *Alytes muletensis* are infected by *B. dendrobatidis* and suffering from chytridiomycosis. We trace the source of this infection by screening archived mortalities from a captive-breeding facility that had been used for re-introduction of the species to its native habitat. Our study provides the first strong evidence that the anthropogenic movement of amphibians is spreading *B. dendrobatidis*; it also provides a salutatory lesson of the need to ensure that breeding-programs are not hot-beds for cross-specific disease transmission, and that species are free of infectious agents prior to re-introduction.

In 1997, mass mortalities of *Alytes obstetricans* in central Spain [6] led to the first report of chytridiomycosis in a wild European amphibian population. In Europe, the pathogen is now known to be widespread ([7] and our unpublished data). The finding of a dead *A. muletensis* juvenile in 2004, and the subsequent confirmation of a *B. dendrobatidis*-positive status prompted us to screen archived captive populations of *A. muletensis* that had been used for re-introductions; to examine the pattern of occurrence of infection in native *A. muletensis* populations; and to determine the multilocus genotypes of isolates of *B. dendrobatidis* from Mallorca relative to other localities worldwide.

Our screening of archived mortalities of captive *A. muletensis* using whole-genome amplification, quantitative PCR [8] and histology found clear evidence of *B. dendrobatidis* in 1991, 1992, 1994 and 1995 (Figure S1 and Table S1 in the Supplemental data available on-line). *Xenopus gilli*, an endangered frog endemic to the Western Cape, South Africa, was brought into the same breeding facilities in 1991 and housed in the same room as the *A. muletensis* colony. Of the two specimens of *X. gilli* mortalities examined, one was positive for *B. dendrobatidis*. Following the import of *X. gilli*, 23 captive *A. muletensis* died. Of the five *A. muletensis* mortalities archived in 1991, three were positive for *B. dendrobatidis.*

Surveillance of wild populations in Mallorca found that *B. dendrobatidis* was present in four of the 21 populations surveyed. The distribution of *B. dendrobatidis* among the larval populations was heterogeneous and highly clustered (Potthoff-Whittinghill test statistic, $p < 0.001$; Figure 1; Table S2 in the Supplemental data). In two of these infected populations, Cocó de sa Bova and Torrent des Ferrerets, a prevalence of, or almost, 100% was recorded. The population at Cocó de sa Bova received animals from the aforementioned breeding facility in 1991. Multilocus genotypes from five Mallorcan isolates of *B. dendrobatidis* were all identical to each other, and different to those known from both mainland Spain and the UK (Figure 2). This finding is consistent with there having been a single introduction of an exotic strain of *B. dendrobatidis* into Mallorca. Taking into account the heterogeneous distribution of infection on Mallorca, the presence of *B. dendrobatidis* in both the captive *A. muletensis* and *X. gilli*, and the lack of similarity between the identical genotypes of *B. dendrobatidis* from Mallorca compared to those from mainland Spain and elsewhere, we believe that a recent introduction of *B. dendrobatidis* to

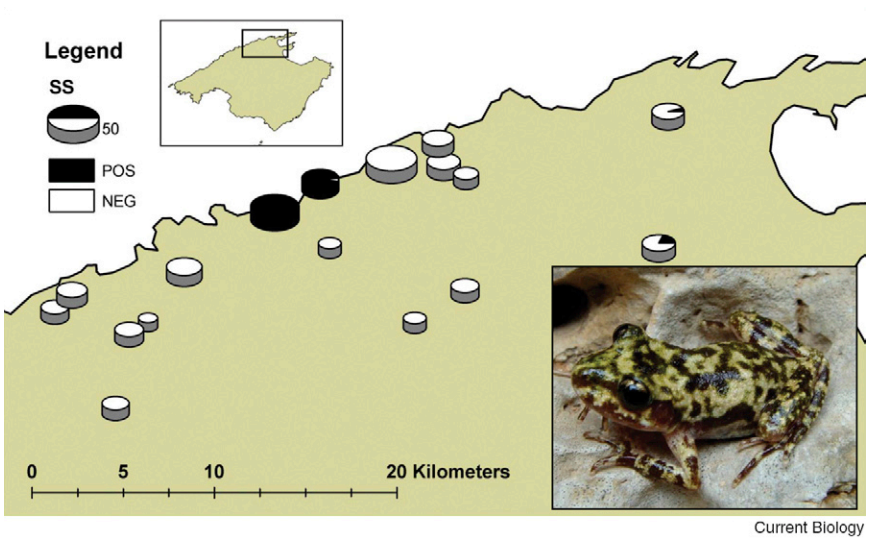

Figure 1. The distribution of surveillance sites in the Sierra de Tramuntana, Mallorca (inset) and prevalence of *B. dendrobatidis*. The samples size (SS) at each locality is indicated by the size of the pie-chart. In the legend a locality with a sample size of 50 is shown. The negative sites are indicated in white. The study species, the Mallorcan Midwife Toad *Alytes muletensis* is shown.

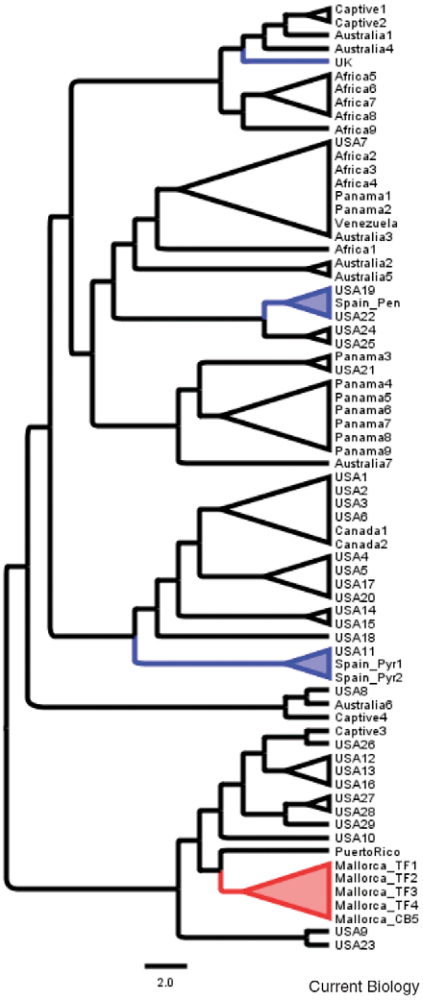

Figure 2. *B. dendrobatidis* infection of *A. muletensis*.

Neighbour-Joining tree of Mallorcan isolates (red), other European isolates (blue) and isolates from the rest of the world (black). The source of the isolates from Mallorca is indicated by TF for Torrent des Ferrerets and CB for Cocó de sa Bova. Numbers of nucleotide substitutions are shown by the scale bar.

Mallorca vectored by the species re-introduction program has occurred. However, the lack of phylogeographic structure for *B. dendrobatidis* makes identifying the source of this introduction impossible without further global sampling.

Our results reinforce the need to follow the IUCN guidelines for captive breeding. To avoid disease transmission, stringent screening, quarantine periods and single-species facilities are required. Across taxa, disease may be a significant threat if wild animals are infected prior to the time of capture, if infection is acquired in captivity (as in the case of *A. muletensis*), or if infection is acquired post-release. For example, the Sharp-snouted day frog, *Taudactylus acutirostris*, an Australian endemic thought to be extinct in the wild due to chytridiomycosis, latterly became extinct in captivity as a result of infection which might have been acquired prior to the time of capture [9]. In contrast, in the case of the Mauritius pink pigeon *Columba mayeri*, avian trichomonosis has been acquired post-release. The occurrence of *Trichomonas gallinae* may be traced back to the introduction of the Rock pigeon, *Collumba livea* [10].

In the case of the Mallorcan midwife toad, efforts to screen for, and mitigate against, infections in the captive collection were overshadowed by the undetected presence of *B. dendrobatidis*, at a time which predated the discovery of this pathogen by seven years. Following the listing of *B. dendrobatidis* as a notifiable organism by the OIE [5] and the current onus on captive breeding by the IUCN amphibian specialist group, routine procedures for the treatment and testing of amphibians, and the use of biosecure facilities are becoming mandatory. These protocols are necessary to stem the further introduction of this pathogen into disease-free regions. Consequently, there is a need for country-wide surveys to determine the infection-status of both native and introduced amphibians in order to inform policy-makers. Together, these are key tools for addressing the global spread of *B. dendrobatidis* and managing the future emergence of this most destructive of pathogens.

Supplemental Data

Supplemental data are available at http://www.current-biology.com/cgi/content/full/18/18/R853/DC1

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