Climate change promotes hybridisation between deeply divergent species of European toads

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Abstract

Rare hybridisations between deeply divergent animal species have been reported for decades in a wide range of taxa, but have often remained unexplained, mainly regarded as chance events and, as such, reported as anecdotal. Here, we combine field observations with long-term data concerning natural hybridizations, climate, land-use, and field-validated species distribution models, for two deeply divergent, naturally sympatric, and occasionally interbreeding toad species in Europe, belonging to the *Bufo bufo* and *Bufotes viridis* species groups. We show that climate warming and seasonal extreme temperatures are conspiring to set the scene for these maladaptive hybridisations, by differentially affecting life-history traits of both species. Our results identify and provide evidence of an ultimate cause for such events, and reveal that the potential influence of climate change on interspecific hybridisations goes far beyond closely related species. Furthermore, climate projections suggest that the chances for these events will steadily increase in the near future.

Running head: Climate change promotes maladaptive hybridisations

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Introduction

Hybridisation is a widespread phenomenon in nature (Mallet, 2005), yet the frequency, diversity of outcomes, underlying mechanisms, its role in the evolutionary process, and how to deal with it in conservation biology have been controversial topics for more than a century (Arnold, 2006; Schwenk et al., 2008). Much of our knowledge about the link between hybridisation dynamics in animals and climate changes, comes from studies of hybrid zones (Hewitt, 2011), where the reshuffling of species’ ranges in response to changing climates brought into contact closely related and previously allopatric species. Pre-mating reproductive barriers could be incomplete between these species, and their genomes be still porous to introgression of foreign material, with several far reaching implications (Mallet, 2005; Arnold, 2006; Schwenk et al., 2008; Hewitt, 2011). Understandably, species of ancient divergence and with a long-lasting history of coexistence have contributed the least to this body of knowledge (Mallet, 2005; Schwenk et al., 2008). These species have had ample opportunity to evolve strong pre-mating reproductive barriers, either as a by-product of a longer allopatric divergence or because of character displacement in response to natural selection to lessen reproductive interaction and/or resource competition (Coyne, 2004; Pfenning and Pfenning, 2009). Consequently, hybridisation events are extremely improbable between these species, and their observation incidental in the wild.

We witnessed to one such events in southern Italy between two toad species, the common toad *Bufo bufo* and the green toad *Bufotes balearicus* (Figure 1). They belong to the *Bufo bufo* and *Bufotes viridis* species groups, whose divergence has been estimated to the Oligocene (around 20-30 million years ago; Maxson, 1981; Garcia-Porta et al., 2012), and which have largely overlapping distributions in central, eastern, and southern Europe (Sillero et al., 2014). Although syntopy is not uncommon, especially in lowland areas, they show distinct spatio-temporal patterns of habitat use (reviewed in Lanza et al., 2006), making our finding at least
three-times unexpected. First, they show markedly different breeding phenologies: *B. bufo* is an early and explosive breeder, while *B. viridis* is a late and prolonged breeder. Along the Italian peninsula, breeding activities of *B. bufo* begin earlier in the year (early winter to early spring, with variation among sites at different altitude and latitude) and usually last 1–2 weeks. *B. viridis* starts breeding later (middle to late spring), and this activity may last 2–3 months. Furthermore, breeding activities in syntopic areas have been systematically reported as allochronic, regardless of intraspecific differences between sites. Second, *B. bufo* and *B. viridis* display differences in their altitudinal distribution. *B. bufo* breeding sites commonly occur from 0 to 2000 m above sea level (MASL), while *B. viridis* shows marked preferences for sites in lowland areas, rarely being observed above 1000 MASL. Third, in spite of their largely polytopic habits, differences exist in habitat and breeding site preferences. *B. viridis* favours open areas and bushlands and breeds in temporary shallow waters, and *B. bufo* most commonly inhabits forested habitats while using slow running or deeper, wider standing waters as breeding sites.

Remarkably, before our observation (May 2014), all these differences applied to toad populations at our study site. This site is a high-altitude pond (1330 MASL) located at the margins of a forested area (Figure 2). *B. viridis* was absent at this location and in neighbouring areas above 800 MASL at least until the year 2007 (Carpino and Capasso, 2008), while breeding activities of the resident *B. bufo* population were recorded on February of the same year (Carpino and Capasso, 2008).

Here we combine our observation with data from previous reports of similar events in Europe along the last century. To study the causation of these ‘improbable’ hybridisations, we adopted an integrative experimental approach, and examined the contribution of multiple factors, including all those commonly invoked to explain novel interspecific hybridisations among animal species in the wild.
Materials and Methods

Assessing natural hybridisation

We visually searched for additional evidence of hybridisation at the breeding pond, for the ten days immediately following our initial observation of the hybrid couple. Although no further heterospecific couples were observed, two newly laid egg-strings were found.

In order to confirm the hybrid nature of the egg-string laid by the hybrid couple (against the hypotheses of unfertilised eggs and of undetected homospecific paternity) and to address the parental species of the other egg-strings, we monitored egg and tadpole development under laboratory conditions and analysed the pattern of variation of individual larvae at diagnostic genetic markers.

A strip approximately 1.5 m in length was removed from each egg-string and transported to the laboratory. Tadpoles were reared under standardised light and food conditions, in plastic boxes (0.8 × 0.5 × 0.2 m) filled with oxygenated tap water. Larval mortality was checked twice daily, from hatching to metamorphosis.

Tadpoles of \textit{B. bufo} and \textit{B. viridis} can be distinguished based on larval morphology (Ambrogio and Mezzandri, 2014); hybrid tadpoles are usually heavily malformed (Montalenti, 1933; Montalenti, 1932). However, in order to achieve correct identification and to verify the absence of backcrosses between hybrids and parental individuals, we analysed genetic variation at the following allozyme loci: malate dehydrogenase (Mdh-1 and Mdh-2; EC 1.1.1.37), isocitrate dehydrogenase (Icdh-1 and Icdh-2; EC 1.1.1.42), and malate dehydrogenase NADP+-dependent (Mdhp-1; EC 1.1.1.40). Fifty tadpoles from each egg-string were killed using a 200 mg/L solution of MS222, 10 days after hatching, and then stored at -80°C for subsequent analyses. The diagnostic value of each allozyme locus was verified through preliminary analyses.
of 20 individuals per species, sampled from two sites in neighbouring areas, where no evidence of potential hybridisation had been observed (B. *bufo*: 41.1737° N, 14.5834° E; B. *viridis*: 40.8866° N, 14.9318° E). Standard horizontal starch gel electrophoresis and zymogram visualisation procedures were carried out, following previously published standard protocols (Harris and Hopkinson, 1976).

**Quantifying land cover change**

In order to assess land cover change as a possible explanation for the hybridisation event registered in May 2014, in southern Italy, we performed a land use change analysis considering an area of 119 km², inside the Partenio Regional Park (Regione Campania, Italy). The clearing where the hybridisation event was registered is located within the internal part of the study area, approximately 15.6 km from the northern boundary and 2 km from the south-eastern boundary (Figure 2). A map of the area was sourced using Google Earth Pro 7.1.5.1557 (Google Inc., Mountain View, California). By using the “historical imagery” tool, and keeping the extent and resolution of the map constant, we selected two images: one from 10 October 2014, a few months after the hybridisation event, and one from 31 October 2006, a few months before the last record indicating an absence of B. *viridis* from the site (Carpino and Capasso, 2008). The two images were imported into ArcGIS 10.3.1 (ESRI ©), with a resolution of 4.5 m per pixel, and were georeferenced using administrative boundaries as reference points (RMS error = 6.23 m for the 2014 image; RMS error = 4.48 m for the 2006 image). The images were interpreted using direct recognition (Campbell, 1978), considering five easily recognisable land use classes that hold a clear ecological importance for both toad species: agriculture, forests, natural clearings, natural vegetation (other than forests), and artificial. For both images, a vector layer (format shapefile, ESRI ©) was produced at a 1:25,000 scale. To perform the land cover change
analysis, as per (Falcucci et al., 2007), each vector layer was transformed into a raster layer considering 4 different pixel resolutions: 25 m, 50 m, 75 m, and 100 m. The change detection analysis was performed for each pixel resolution, resulting in an average percentage change for every land cover class.

**Climate influence on altitudinal distribution pattern**

Based on historical records of occurrence, and considering the species’ altitudinal distributions in peninsular Italy (Carpino and Capasso, 2008; Guarino et al., 2012; Lanza et al., 2007), the presence of a *B. bufo* population at the study site was an expected finding, whereas the presence of *B. viridis* was not expected, either at this site or within neighbouring, high altitude areas. Therefore, we focused subsequent analyses on the latter species.

To address the plausibility of climate forcing on recent altitudinal distribution changes for *B. viridis*, we calibrated a correlative distribution model for the species in peninsular Italy. The models were calibrated considering six bioclimatic variables considered important for the species: temperature seasonality, mean temperature of the warmest quarter, mean temperature of the coldest quarter, temperature annual range, precipitation seasonality, and precipitation of the coldest quarter. We obtained all climate variables at 1 km resolution from WORLDCLIM (Hijmans et al., 2005), which provide an interpolation of climate observation representative of 1950–2000. To obtain the average climate variables for the 2007–2013 time frame, we followed the procedure presented in (Maiorano et al., 2013) and considered the data available from the Climatic Research Unit of the University of East Anglia (database: CRU TS3.22; Harris et al., 2014), which provided monthly temperature and precipitation values with a spatial resolution of 50 × 50 km. To downscale from the resolution of the CRU database to the 1 km² of the WORLDCLIM database, we first calculated climate anomalies by contrasting monthly
temperature and precipitation values for 2007–2013 against the 1950–2000 climate data, as obtained from the same CRU TS3.22 database. Anomalies were calculated as absolute temperature difference (Δ°C) and relative precipitation differences (% change). By using bilinear resampling, we downscaled the anomalies to 0.0083° of spatial resolution (≈ 1-km). Then, in order to obtain monthly maps of temperature and precipitation for 2007–2013, we applied the anomaly corrections to the 1950–2000 climate layers. Finally, we calculated all the derived climate maps mentioned above. The bioclimatic layers for 2070–2100 were obtained directly at the resolution of 1 km² from the WORLDCLIM database, and considered 3 emission scenarios (A1B, A2, and B1), and many different global circulation models (24 GCMs for the A1B emission scenario, 19 GCMs for A2, and 18 GCMs for B1) developed under IPCC4 (IPCC, 2007).

To calibrate the models, we used the ensemble forecasting approach (Araújo and New, 2007) implemented in BIOMOD, a bioclimatic niche modelling package for the R environment (Thuiller et al., 2009). We used the following eight models: (i) generalised linear models, (ii) generalised additive models, (iii) classification tree analysis, (iv) artificial neural networks, (v) generalised boosted models, (vi) random forests, (vii) flexible discriminant analysis, and (viii) multivariate adaptive regression spline. All models were calibrated considering the entirety of peninsular Italy south of the Po river (212,460 km²), with 350 points of presence collected before 2000, plus 10,000 background points. All models were evaluated using a repeated split-plot procedure (70% of the data used for calibration, 30% left apart for evaluation; the entire procedure repeated 10 times for each model (Thuiller et al., 2009), and by measuring the area under the receiver operating characteristic (ROC) curve (AUC) (Swets, 1988). All models with AUC values greater than 0.745 were projected over the entire study area using the 1950–2000 climate layers, as well as the 2007–2013, and 2070-2100 climate layers. We measured the
minimum probability of presence obtained in correspondence of the available points of presence for 1950–2000, and we used this threshold to define areas of species absence (all areas below this minimum threshold) in all periods considered. Moreover, considering 100 m wide elevation classes, we calculated average probability of presence for all three periods, and obtained a model of the probability of presence for *B. viridis* along the elevation range in peninsular Italy (Figure 3).

We further investigated the plausibility of a link between climate change and altitudinal shifts by turning the model prediction into a working hypothesis. Based on this prediction (see Results and Discussion), we selected a geographic area close to our study site, and carried out field searches for further, unprecedented sites of occurrence of *B. viridis*, above 1200 MASL. To select the geographic area, we adopted the following criteria: (i) location on a mountain massif, as close as possible to our study site; (ii) presence of potential breeding sites at altitudes ≥1200 MASL; (iii) *B. viridis* populations reported at lower altitudes; (iv) absence of obvious anthropogenic habitat discontinuities between low and high altitude areas. Accordingly, we identified the Picentini Mountains (within the Picentini Mountains Regional Park, roughly located 25 Km southeast of our study site) as an area of best fit for our criteria. Field searches began on 2 May 2015, and lasted until the first evidence of *B. viridis* in the area was found (21 May). The rationale underlying this experimental integration was as follows: although failure to identify new high altitude sites of occurrence would not be strong evidence against a role of climate change in promoting altitudinal shifts at lake Campo Maggiore, or elsewhere, a positive result would provide support for the model prediction, and therefore, support the hypothesis that our initial finding belongs to a suite of events promoted by climate change.

**Climate influence on breeding phenology**
Our finding of the hybrid couple in May 2014 suggests delayed breeding activity of *B. bufo* within the usual breeding period of *B. viridis* (Lanza et al., 2006; Carpino and Capasso, 2008; Guarino et al., 2012), a pattern shared by most previous observation (Vlček, 1997; Lang, 1926; Vlček, 1995; Zavadil and Roth, 1997; Duda, 2008). Therefore, subsequent analyses were focused on *B. bufo*. Notably, while there is strong evidence for a link between the breeding phenology of *B. bufo* and annual temperature cycles (Reading, 1998; Reading, 2003; Tryjanowski et al., 2003), the same does not hold true for *B. viridis*.

A search of academic and grey literature revealed five additional observations of hybrid couples, at four geographic sites (see Figure 3): two located in the Czech Republic (Vlček, 1997; Lang, 1926; Vlček, 1995; Zavadil and Roth, 1999), one in Sweden (Lang, 1926), and one in Austria (Duda, 2008).

The annual activity cycle of *B. bufo* populations can be affected by several environmental features, including climate, and the five sites (including our observation) span a wide range of latitudes. Thus, rather than considering average winter temperatures, we based our analysis on the period when this species begins its breeding activity in each area, according to regional atlases and databases (Guarino et al., 2012; Nečas et al., 1997; Cabela and Grillitsch, 1997; Gilsen and Kauri, 1959). In addition, previous studies suggested that the beginning of this activity is linked to the average temperatures of the preceding 1–2 months (Reading, 1998). Therefore, in our testing for a link between hybridisation events and climate anomalies, we set the period of interest to the two months preceding the usual start of the breeding activity, for each geographic area. Accordingly, we analysed date ranges covering December to January for the site in south-central Italy, January to February for the sites in Czech Republic and Austria, and February to March for the site in Sweden.
Long-term climate data for our study site were provided, by the Montevergine Observatory (40.9360° N; 14.7288° E), as monthly averages since the year 1884. In order to gain climate data for the four sites of past hybridisation, we searched the NOAA database (available at http://gis.ncdc.noaa.gov) of monthly observational data using the following two criteria: (i) climate station closest to the site of interest and (ii) time series of at least 40 years before the year of the observed hybridisation event. The following stations best matched these search criteria: Koebenhavn Landbohojskolen, Denmark (Id: DA000030380; Latitude: 55.683° N; Longitude: 12.533° E); Praha Klementinum, Czech Rep. (Id: EZE00100082; Latitude: 50.090° N; Longitude: 14.419° E); Wien, Austria (Id: AU000005901; Latitude: 48.233° N; Longitude: 16.35° E); Oravska Lesna, (Id: LOE00116364; Latitude: 49.366° N; Longitude: 19.166° E).

For each climatic series retrieved, we analysed bimonthly average temperatures along both the entire temporal series, and the 10 years preceding the hybridisation event, i.e. a time-lapse approximating the lifetime climatic experience of the ‘average’ toad in the wild (Lanza et al., 2006).

To test the null hypothesis that an association between hybridisation events and climate anomalies was due to chance alone, we carried out binomial probability tests. We set the probability threshold of a single event to 0.02, based on the highest value calculated for the ratio between year rank (mildest = 1st rank) and climatic series length (i.e. the first out of 47 available years from the climatic station DA000030380). Since hybridisation events were both spatially and temporally distant, data independence was assumed. However, to err on the side of caution, we carried out the analyses considering the two observations in eastern Czech Republic, as both independent and fully dependent (i.e. as a single observation), then took the highest value as the confidence level for accepting/rejecting the null hypothesis.
Finally, the paucity of hybridisation events recorded qualifies these events as rare, and testifies to the strength of the pre-mating isolation mechanisms. On the other hand, given such rareness, we cannot exclude the occurrence of potentially unobserved, unreported, or undetected events. Thus, we explored how potentially unrecorded events could affect the significance of our test. To this aim, we carried out additional binomial probability tests by progressively increasing the number of events while leaving the number of ‘successes’ unchanged. The null hypotheses of no association was rejected at the nominal probability threshold $\alpha = 0.05$.

**Results and Discussion**

At the time of our observation, we counted 9 males, 3 females and 8 juveniles (22-26 mm long; presumably 1 year old) of *B. viridis*, plus 2 female *B. bufo*, and various newly spawned egg-strings. Tadpoles from the putatively hybrid egg-string were identified as first-generation hybrids by their heterozygote status at all loci analysed. All other individuals analysed were homozygotic for the respective diagnostic allele at each locus, and were thus identified as belonging to parental species. Therefore, no further data analysis was carried out on the multilocus genotypes. In line with previous findings (Montalenti, 1933; Montalenti, 1932), most hybrid tadpoles were heavily malformed (see Figure 1), and none reached the metamorphosis, whereas tadpoles from homospecific pairs did not show abnormalities, neither in the external morphology nor in the ontogenetic pathway.

Despite their wide sympatry, ease of observation, and more than a century-old knowledge of hybridisation in laboratory crosses, our literature searches for previous reports of interspecific breeding couples in the wild, identified just 5 additional observations within a 94-year time span (Vlček, 1997; Lang, 1926; Vlček, 1995; Zavadil and Roth, 1997; Duda, 2008).
Three main hypotheses have been invoked to explain recently established interspecific hybridisations among animal species, and may have played a role in the present case by promoting syntopy and breeding season overlap (Chunco, 2014; Crispo et al., 2011): species translocations, anthropogenic habitat degradation (a derivation of the Anderson’s ‘hybridisation of the habitat’ model; Anderson, 1948) and climate changes.

In the case of *B. bufo* and *B. viridis*, a species translocation can be firmly excluded in all the reported cases, based on the extensive knowledge of their natural geographic distributions (Sillero et al., 2014; Lanza et al., 2006), as well as on the fossil data of both species in Europe (Martin and Sanchiz, 2011).

Anthropogenic habitat degradation has been proposed as a main causative agent in some case (Duda, 2008). By reducing the diversity and number of potential breeding sites in a given area, physical alterations of habitat could promote syntopy of previously allotopic populations of *B. bufo* and *B. viridis*. Although plausibly contributing, this hypothesis cannot explain the entire pattern, and it does not apply to all cases. Our study site (but see also Zavadil and Roth, 1997) is located within a protected area established in 1993, and an analysis of contemporary and historical aerial photos of this site and neighbouring areas clearly show the absence of any physical alterations of potential relevance for the two species (Figure 2). Moreover, habitat degradation could not explain the overlap of the two breeding seasons. Climate changes, however, significantly improve our ability to explain the occurrence of hybridisation events between these species.

By promoting a recent altitudinal migration of *B. viridis* from neighbouring, lower altitude sites, the ongoing climate warming engendered the unexpected syntopy at our study site. Support this argument (the only alternative to recent translocation), comes from our models of the distribution of *B. viridis* in peninsular Italy, based on a set of known occurrences collected before
year 2000, and projected to the average climate over the period 2007-2013. Indeed, our models indicated that the species’ presence above 1200 MASL was highly improbable under pre-2000 climate, but became plausible during 2007-2013 (Figure 3). Furthermore, projecting the models under future climate projections for the time period 2070-2100 under different emission scenarios the general pattern remains unchanged, but with an even higher probability of presence above 1200 MASL (results not shown). The reliability of the models was clearly confirmed by the field-validation procedure (see Methods). Indeed, our field searches of *B. viridis* at high-altitude sites of predicted presence in post-2000 projections were successful. We found a previously unreported site of occurrence within the Picentini Mountains (Latitude: 40.8251°N; Longitude: 14.9864°E; roughly 25 Km south-east of the study site), thus confirming that upward migrations of *B. viridis* are ongoing, as predicted by our bioclimatic model (see also Zavadil and Roth, 1997).

Besides being a co-factor, priming syntopy, climate changes also contributed to hybridisation events by promoting an overlap of the breeding activities. Analysing long-climate series, we found that the years when hybridisation events were observed in Europe (including our observation) ranked 1st or 2nd hottest on record at each site, over time series from 47 to 214 years-long. Moreover, bimonthly mean temperatures at these sites were 2.4 to 5.5 °C above the 1961-1990 averages, and 1.9 to 4.3 °C above the preceding 10-years averages (Figure 3). Binomial probability tests allowed us to reject the null hypothesis of random association between hybridisation events and extremely mild winters, with very high confidence (binomial probability: \( P \leq 2.3 \times 10^{-6} \)). Also, additional binomial probability tests, carried out in order to explore how unrecorded events could affect the significance of our test, indicated that the null hypothesis of random association was rejected (at \( \alpha = 0.05 \)) until the number of events was \( \geq 77 \), while leaving unchanged the number of ‘successes’.
Although *B. bufo* is expected to bring forward its breeding activity after mild winters (Reading, 1998; Reading, 2003; Tryjanowski et al., 2003), at least three lines of support may help explaining this apparently counterintuitive pattern. In years when the breeding season begins earlier (after a mild winter), breeding has been observed to last longer (Gittins et al., 1980). Furthermore, a second and lower peak of breeding activity has been often observed later in the season (Reading, 1998; Pages, 1984), especially after mild winters (Reading, 1998). Finally, extensive ecophysiological investigations on bufonid toads, including *B. bufo*, indicate that increased temperatures during hibernation lead to significant alterations of several processes affecting the breeding activity, including body size condition, annual ovarian cycle, and seasonal synchronisation of breeding (Jørgensen, 1992).

Our analyses do not indicate climate change as the single explanatory factor. The environmental contexts in which interspecific interactions occur and the diverse forms of habitat disturbance might be locally influential. Nonetheless, these analyses clearly show that climate changes played a fundamental part in promoting hybridisation events. In light of the direction of these changes (IPCC, 2014), and of the results of our modelling exercise, we hypothesise that these events will become progressively more common in the near future. Most importantly, our results reveal a wider potential influence of climate changes on interspecific reproductive interactions, particularly in the many instances where climate-driven allochrony and/or allotopy are integral components of the reproductive isolating barriers.

Hybridisation events among non-closely related species are generally believed to yield events that are transient, and potentially affecting local population demography at most, because strongly maladaptive (Malone and Fontnot, 2008; Rhymer and Simberloff, 1996). Nevertheless, there may be exceptions, whereby the effects of maladaptive processes propagate from population to community level (Farkas, 2015). Moreover, as revealed by years of investigation
on the hybridisation process in several animal taxa, including amphibians, new evolutionary pathways have been sometime opened by such rare and maladaptive events (Arnold, 2006).
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Conflict of interest

The authors have no conflict of interests to declare.
Figure 1. Interspecific hybridisation between the common toad (*Bufo bufo*) and the Balearic green toad (*Bufotes balearicus*) in the wild. The hybrid couple (a) was found spawning (b) on 10 May 2014, at lake Campo Maggiore, a high-elevation pond within the Partenio Regional Park, southern Italy (latitude: 40.9429° N; longitude: 14.7096° E; altitude: 1330 MASL). The majority of tadpoles from the hybrid egg-string reared under standard laboratory conditions were heavily malformed (inset), and none survived until the metamorphosis stage; this pattern was not observed for control tadpoles from co-specific mates of both species.
Figure 2. Land-use change detection analysis. (a) Location of the study area in Italy. (b) Map of the land use in 2006, as obtained from direct interpretation of an aerial photo collected on 31 October 2006; in the same map, the exact location where the hybridisation event has been registered is indicated, as well as all areas where land use was different when compared to a second aerial photo collected on 9 October 2014. Both aerial photos were obtained from Google Earth Pro 7.1.5.1557 (Google Inc., Mountain View, California). (c) Average percent change (range of percent change in parenthesis) in land use classes from 2006 to 2014; total km² area for each land cover class in 2006 is provided in the last column. Aerial photos of the breeding site and its neighbourhoods collected on 31 October 2006 (d), and 9 October 2014 (e).
Figure 3. Climate correlates of the interspecific hybridisation events observed in the wild between the common toad (*Bufo bufo*) and the Balearic green toad (*Bufotes balearicus*). Bar plots showing frequency distribution (percent) of bimonthly mean temperature deviations (ΔTm) from the 1961–1990 average, compared to the two months preceding the breeding activity at each geographic region: (a) December to January, (b) February to March, and (c-e) January to February. Values for the years when hybrid mates were observed are marked using red arrows. Optimal bar width was computed for each climatic series following the Freedman–Diaconis rule. (f) Average probability of presence vs elevation at sea level (m) as modelled for the pre-2000 climate (red line), and for the 2007–2013 climate (solid black line); the black dotted line indicates the minimum plausible level of probability of presence, above which the species can be considered present, while below is considered absent. (g) Mean temperature data for each site, and year of observation of interspecific mates. CsL: climatic series length, in years, before the observed event; b-MT: bimonthly mean temperature; R: rank over the entire climatic series (1= mildest); ΔTm: deviation from the 1961–1990 average temperature (°C); ΔT10y: deviation from the preceding 10-year average temperature (°C).