

1 **Climate change promotes hybridisation between deeply divergent**
2 **species of European toads**

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17

18 **Abstract**

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

31

32 **Running head:** Climate change promotes maladaptive hybridisations

33 **Keywords:** Hybridisation, Climate change, Life-history traits, Pre-mating reproductive barriers.

34

35 **Introduction**

36 Hybridisation is a widespread phenomenon in nature (Mallet, 2005), yet the frequency, diversity
37 of outcomes, underlying mechanisms, its role in the evolutionary process, and how to deal with it
38 in conservation biology have been controversial topics for more than a century (Arnold, 2006;
39 Schwenk et al., 2008). Much of our knowledge about the link between hybridisation dynamics in
40 animals and climate changes, comes from studies of hybrid zones (Hewitt, 2011), where the
41 reshuffling of species' ranges in response to changing climates brought into contact closely
42 related and previously allopatric species. Pre-mating reproductive barriers could be incomplete
43 between these species, and their genomes be still porous to introgression of foreign material,
44 with several far reaching implications (Mallet, 2005; Arnold, 2006; Schwenk et al., 2008;
45 Hewitt, 2011). Understandably, species of ancient divergence and with a long-lasting history of
46 coexistence have contributed the least to this body of knowledge (Mallet, 2005; Schwenk et al.,
47 2008). These species have had ample opportunity to evolve strong pre-mating reproductive
48 barriers, either as a by-product of a longer allopatric divergence or because of character
49 displacement in response to natural selection to lessen reproductive interaction and/or resource
50 competition (Coyne, 2004; Pfenning and Pfenning, 2009). Consequently, hybridisation events
51 are extremely improbable between these species, and their observation incidental in the wild.

52 We witnessed to one such events in southern Italy between two toad species, the common
53 toad *Bufo bufo* and the green toad *Bufo balearicus* (Figure 1). They belong to the *Bufo bufo*
54 and *Bufo viridis* species groups, whose divergence has been estimated to the Oligocene
55 (around 20-30 million years ago; Maxson, 1981; Garcia-Porta et al., 2012), and which have
56 largely overlapping distributions in central, eastern, and southern Europe (Sillero et al., 2014).
57 Although syntopy is not uncommon, especially in lowland areas, they show distinct spatio-
58 temporal patterns of habitat use (reviewed in Lanza et al., 2006), making our finding at least

59 three-times unexpected. First, they show markedly different breeding phenologies: *B. bufo* is an
60 early and explosive breeder, while *B. viridis* is a late and prolonged breeder. Along the Italian
61 peninsula, breeding activities of *B. bufo* begin earlier in the year (early winter to early spring,
62 with variation among sites at different altitude and latitude) and usually last 1–2 weeks. *B. viridis*
63 starts breeding later (middle to late spring), and this activity may last 2–3 months. Furthermore,
64 breeding activities in syntopic areas have been systematically reported as allochronic, regardless
65 of intraspecific differences between sites. Second, *B. bufo* and *B. viridis* display differences in
66 their altitudinal distribution. *B. bufo* breeding sites commonly occurs from 0 to 2000 m above sea
67 level (MASL), while *B. viridis* shows marked preferences for sites in lowland areas, rarely being
68 observed above 1000 MASL. Third, in spite of their largely polytopic habits, differences exist in
69 habitat and breeding site preferences. *B. viridis* favours open areas and bushlands and breeds in
70 temporary shallow waters, and *B. bufo* most commonly inhabits forested habitats while using
71 slow running or deeper, wider standing waters as breeding sites.

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

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

83 **Materials and Methods**

84

85 **Assessing natural hybridisation**

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

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

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

98 Tadpoles of *B. bufo* and *B. viridis* can be distinguished based on larval morphology
99 (Ambrogio and Mezzandri, 2014); hybrid tadpoles are usually heavily malformed (Montalenti,
100 1933; Montalenti, 1932). However, in order to achieve correct identification and to verify the
101 absence of backcrosses between hybrids and parental individuals, we analysed genetic variation
102 at the following allozyme loci: malate dehydrogenase (Mdh-1 and Mdh-2; EC 1.1.1.37),
103 isocitrate dehydrogenase (Icdh-1 and Icdh-2; EC 1.1.1.42), and malate dehydrogenase NADP+-
104 dependent (Mdhp-1; EC 1.1.1.40). Fifty tadpoles from each egg-string were killed using a 200
105 mg/L solution of MS222, 10 days after hatching, and then stored at -80°C for subsequent
106 analyses. The diagnostic value of each allozyme locus was verified through preliminary analyses

107 of 20 individuals per species, sampled from two sites in neighbouring areas, where no evidence
108 of potential hybridisation had been observed (*B. bufo*: 41.1737° N, 14.5834° E; *B. viridis*:
109 40.8866° N, 14.9318° E). Standard horizontal starch gel electrophoresis and zymogram
110 visualisation procedures were carried out, following previously published standard protocols
111 (Harris and Hopkinson, 1976).

112

113 **Quantifying land cover change**

114 In order to assess land cover change as a possible explanation for the hybridisation event
115 registered in May 2014, in southern Italy, we performed a land use change analysis considering
116 an area of 119 km², inside the Partenio Regional Park (Regione Campania, Italy). The clearing
117 where the hybridisation event was registered is located within the internal part of the study area,
118 approximately 15.6 km from the northern boundary and 2 km from the south-eastern boundary
119 (Figure 2). A map of the area was sourced using Google Earth Pro 7.1.5.1557 (Google Inc.,
120 Mountain View, California). By using the “historical imagery” tool, and keeping the extent and
121 resolution of the map constant, we selected two images: one from 10 October 2014, a few
122 months after the hybridisation event, and one from 31 October 2006, a few months before the
123 last record indicating an absence of *B. viridis* from the site (Carpino and Capasso, 2008). The
124 two images were imported into ArcGIS 10.3.1 (ESRI ©), with a resolution of 4.5 m per pixel,
125 and were georeferenced using administrative boundaries as reference points (RMS error = 6.23
126 m for the 2014 image; RMS error = 4.48 m for the 2006 image). The images were interpreted
127 using direct recognition (Campbell, 1978), considering five easily recognisable land use classes
128 that hold a clear ecological importance for both toad species: agriculture, forests, natural
129 clearings, natural vegetation (other than forests), and artificial. For both images, a vector layer
130 (format shapefile, ESRI ©) was produced at a 1:25.000 scale. To perform the land cover change

131 analysis, as per (Falcucci et al., 2007), each vector layer was transformed into a raster layer
132 considering 4 different pixel resolutions: 25 m, 50 m, 75 m, and 100 m. The change detection
133 analysis was performed for each pixel resolution, resulting in an average percentage change for
134 every land cover class.

135

136 **Climate influence on altitudinal distribution pattern**

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

142 To address the plausibility of climate forcing on recent altitudinal distribution changes for
143 *B. viridis*, we calibrated a correlative distribution model for the species in peninsular Italy. The
144 models were calibrated considering six bioclimatic variables considered important for the
145 species: temperature seasonality, mean temperature of the warmest quarter, mean temperature of
146 the coldest quarter, temperature annual range, precipitation seasonality, and precipitation of the
147 coldest quarter. We obtained all climate variables at 1 km resolution from WORLDCLIM
148 (Hijmans et al., 2005), which provide an interpolation of climate observation representative of
149 1950–2000. To obtain the average climate variables for the 2007–2013 time frame, we followed
150 the procedure presented in (Maiorano et al., 2013) and considered the data available from the
151 Climatic Research Unit of the University of East Anglia (database: CRU TS3.22; Harris et al.,
152 2014), which provided monthly temperature and precipitation values with a spatial resolution of
153 50×50 km. To downscale from the resolution of the CRU database to the 1 km^2 of the
154 WORLDCLIM database, we first calculated climate anomalies by contrasting monthly

155 temperature and precipitation values for 2007–2013 against the 1950–2000 climate data, as
156 obtained from the same CRU TS3.22 database. Anomalies were calculated as absolute
157 temperature difference ($\Delta^{\circ}\text{C}$) and relative precipitation differences (% change). By using bilinear
158 resampling, we downscaled the anomalies to 0.0083° of spatial resolution ($\approx 1\text{-km}$). Then, in
159 order to obtain monthly maps of temperature and precipitation for 2007–2013, we applied the
160 anomaly corrections to the 1950–2000 climate layers. Finally, we calculated all the derived
161 climate maps mentioned above. The bioclimatic layers for 2070–2100 were obtained directly at
162 the resolution of 1 km^2 from the WORLDCLIM database, and considered 3 emission scenarios
163 (A1B, A2, and B1), and many different global circulation models (24 GCMs for the A1B
164 emission scenario, 19 GCMs for A2, and 18 GCMs for B1) developed under IPCC4 (IPCC,
165 2007).

166 To calibrate the models, we used the ensemble forecasting approach (Araújo and New,
167 2007) implemented in BIOMOD, a bioclimatic niche modelling package for the R environment
168 (Thuiller et al., 2009). We used the following eight models: (i) generalised linear models, (ii)
169 generalised additive models, (iii) classification tree analysis, (iv) artificial neural networks, (v)
170 generalised boosted models, (vi) random forests, (vii) flexible discriminant analysis, and (viii)
171 multivariate adaptive regression spline. All models were calibrated considering the entirety of
172 peninsular Italy south of the Po river ($212,460\text{ km}^2$), with 350 points of presence collected before
173 2000, plus 10,000 background points. All models were evaluated using a repeated split-plot
174 procedure (70% of the data used for calibration, 30% left apart for evaluation; the entire
175 procedure repeated 10 times for each model (Thuiller et al., 2009), and by measuring the area
176 under the receiver operating characteristic (ROC) curve (AUC) (Swets, 1988). All models with
177 AUC values greater than 0.745 were projected over the entire study area using the 1950–2000
178 climate layers, as well as the 2007–2013, and 2070–2100 climate layers. We measured the

179 minimum probability of presence obtained in correspondence of the available points of presence
180 for 1950–2000, and we used this threshold to define areas of species absence (all areas below
181 this minimum threshold) in all periods considered. Moreover, considering 100 m wide elevation
182 classes, we calculated average probability of presence for all three periods, and obtained a model
183 of the probability of presence for *B. viridis* along the elevation range in peninsular Italy (Figure
184 3).

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

201

202 **Climate influence on breeding phenology**

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

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

214 The annual activity cycle of *B. bufo* populations can be affected by several environmental
215 features, including climate, and the five sites (including our observation) span a wide range of
216 latitudes. Thus, rather than considering average winter temperatures, we based our analysis on
217 the period when this species begins its breeding activity in each area, according to regional
218 atlases and databases (Guarino et al., 2012; Nečas et al., 1997; Cabela and Grillitsch, 1997;
219 Gilsen and Kauri, 1959). In addition, previous studies suggested that the beginning of this
220 activity is linked to the average temperatures of the preceding 1–2 months (Reading, 1998).
221 Therefore, in our testing for a link between hybridisation events and climate anomalies, we set
222 the period of interest to the two months preceding the usual start of the breeding activity, for
223 each geographic area. Accordingly, we analysed date ranges covering December to January for
224 the site in south-central Italy, January to February for the sites in Czech Republic and Austria,
225 and February to March for the site in Sweden.

226 Long-term climate data for our study site were provided, by the Montevergine Observatory
227 (40.9360° N; 14.7288° E), as monthly averages since the year 1884. In order to gain climate data
228 for the four sites of past hybridisation, we searched the NOAA database (available at
229 <http://gis.ncdc.noaa.gov>) of monthly observational data using the following two criteria: (i)
230 climate station closest to the site of interest and (ii) time series of at least 40 years before the year
231 of the observed hybridisation event. The following stations best matched these search criteria:
232 Kopenhagen Landbohjskolen, Denmark (Id: DA000030380; Latitude: 55.683° N; Longitude:
233 12.533° E); Praha Klementinum, Czech Rep. (Id: EZE00100082; Latitude: 50.090° N;
234 Longitude: 14.419° E); Wien, Austria (Id: AU000005901; Latitude: 48.233° N; Longitude:
235 16.35° E); OravskaLesna, (Id: LOE00116364; Latitude: 49.366° N; Longitude: 19.166° E).

236 For each climatic series retrieved, we analysed bimonthly average temperatures along both the
237 entire temporal series, and the 10 years preceding the hybridisation event, i.e. a time-lapse
238 approximating the lifetime climatic experience of the ‘average’ toad in the wild (Lanza et al.,
239 2006).

240 To test the null hypothesis that an association between hybridisation events and climate
241 anomalies was due to chance alone, we carried out binomial probability tests. We set the
242 probability threshold of a single event to 0.02, based on the highest value calculated for the ratio
243 between year rank (mildest = 1st rank) and climatic series length (i.e. the first out of 47 available
244 years from the climatic station DA000030380). Since hybridisation events were both spatially
245 and temporally distant, data independence was assumed. However, to err on the side of caution,
246 we carried out the analyses considering the two observations in eastern Czech Republic, as both
247 independent and fully dependent (i.e. as a single observation), then took the highest value as the
248 confidence level for accepting/rejecting the null hypothesis.

249 Finally, the paucity of hybridisation events recorded qualifies these events as rare, and
250 testifies to the strength of the pre-mating isolation mechanisms. On the other hand, given such
251 rareness, we cannot exclude the occurrence of potentially unobserved, unreported, or undetected
252 events. Thus, we explored how potentially unrecorded events could affect the significance of our
253 test. To this aim, we carried out additional binomial probability tests by progressively increasing
254 the number of events while leaving the number of ‘successes’ unchanged. The null hypotheses of
255 no association was rejected at the nominal probability threshold $\alpha = 0.05$.

256

257 **Results and Discussion**

258

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

269 Despite their wide sympatry, ease of observation, and more than a century-old knowledge
270 of hybridisation in laboratory crosses, our literature searches for previous reports of interspecific
271 breeding couples in the wild, identified just 5 additional observations within a 94-year time span
272 (Vlček, 1997; Lang, 1926; Vlček, 1995; Zavadil and Roth, 1997; Duda, 2008).

273 Three main hypotheses have been invoked to explain recently established interspecific
274 hybridisations among animal species, and may have played a role in the present case by
275 promoting syntopy and breeding season overlap (Chunco, 2014; Crispo et al., 2011): species
276 translocations, anthropogenic habitat degradation (a derivation of the Anderson's 'hybridisation
277 of the habitat' model; Anderson, 1948) and climate changes.

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

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

293 By promoting a recent altitudinal migration of *B. viridis* from neighbouring, lower altitude
294 sites, the ongoing climate warming engendered the unexpected syntopy at our study site. Support
295 this argument (the only alternative to recent translocation), comes from our models of the
296 distribution of *B. viridis* in peninsular Italy, based on a set of known occurrences collected before

297 year 2000, and projected to the average climate over the period 2007-2013. Indeed, our models
298 indicated that the species' presence above 1200 MASL was highly improbable under pre-2000
299 climate, but became plausible during 2007-2013 (Figure 3). Furthermore, projecting the models
300 under future climate projections for the time period 2070-2100 under different emission
301 scenarios the general pattern remains unchanged, but with an even higher probability of presence
302 above 1200 MASL (results not shown). The reliability of the models was clearly confirmed by
303 the field-validation procedure (see Methods). Indeed, our field searches of *B. viridis* at high-
304 altitude sites of predicted presence in post-2000 projections were successful. We found a
305 previously unreported site of occurrence within the Picentini Mountains (Latitude: 40.8251°N;
306 Longitude: 14.9864°E; roughly 25 Km south-east of the study site), thus confirming that upward
307 migrations of *B. viridis* are ongoing, as predicted by our bioclimatic model (see also Zavadil and
308 Roth, 1997).

309 Besides being a co-factor, priming syntopy, climate changes also contributed to
310 hybridisation events by promoting an overlap of the breeding activities. Analysing long-climate
311 series, we found that the years when hybridisation events were observed in Europe (including
312 our observation) ranked 1st or 2nd hottest on record at each site, over time series from 47 to 214
313 years-long. Moreover, bimonthly mean temperatures at these sites were 2.4 to 5.5 °C above the
314 1961-1990 averages, and 1.9 to 4.3 °C above the preceding 10-years averages (Figure 3).
315 Binomial probability tests allowed us to reject the null hypothesis of random association between
316 hybridisation events and extremely mild winters, with very high confidence (binomial
317 probability: $P \leq 2.3 \times 10^{-6}$). Also, additional binomial probability tests, carried out in order to
318 explore how unrecorded events could affect the significance of our test, indicated that the null
319 hypothesis of random association was rejected (at $\alpha = 0.05$) until the number of events was ≥ 77 ,
320 while leaving unchanged the number of 'successes'.

321 Although *B. bufo* is expected to bring forward its breeding activity after mild winters
322 (Reading, 1998; Reading, 2003; Tryjanowski et al., 2003), at least three lines of support may
323 help explaining this apparently counterintuitive pattern. In years when the breeding season
324 begins earlier (after a mild winter), breeding has been observed to last longer (Gittins et al.,
325 1980). Furthermore, a second and lower peak of breeding activity has been often observed later
326 in the season (Reading, 1998; Pages, 1984), especially after mild winters (Reading, 1998).
327 Finally, extensive ecophysiological investigations on bufonid toads, including *B. bufo*, indicate
328 that increased temperatures during hibernation lead to significant alterations of several processes
329 affecting the breeding activity, including body size condition, annual ovarian cycle, and seasonal
330 synchronisation of breeding (Jørgensen, 1992).

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

340 Hybridisation events among non-closely related species are generally believed to yield
341 events that are transient, and potentially affecting local population demography at most, because
342 strongly maladaptive (Malone and Fontnot, 2008; Rhymer and Simberloff, 1996). Nevertheless,
343 there may be exceptions, whereby the effects of maladaptive processes propagate from
344 population to community level (Farkas, 2015). Moreover, as revealed by years of investigation

345 on the hybridisation process in several animal taxa, including amphibians, new evolutionary
346 pathways have been sometime opened by such rare and maladaptive events (Arnold, 2006).

347

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456 Societas Europaea Herpetologica, Bonn.
- 457

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462

463 **Conflict of interest**

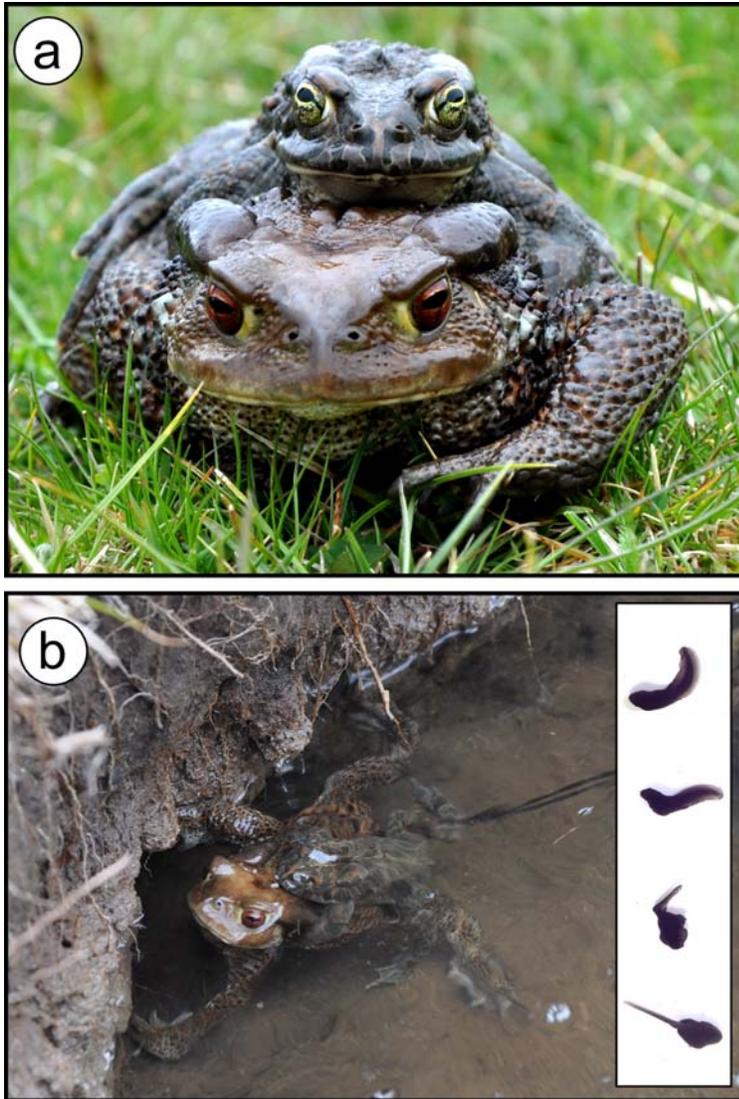
464 The authors have no conflict of interests to declare.

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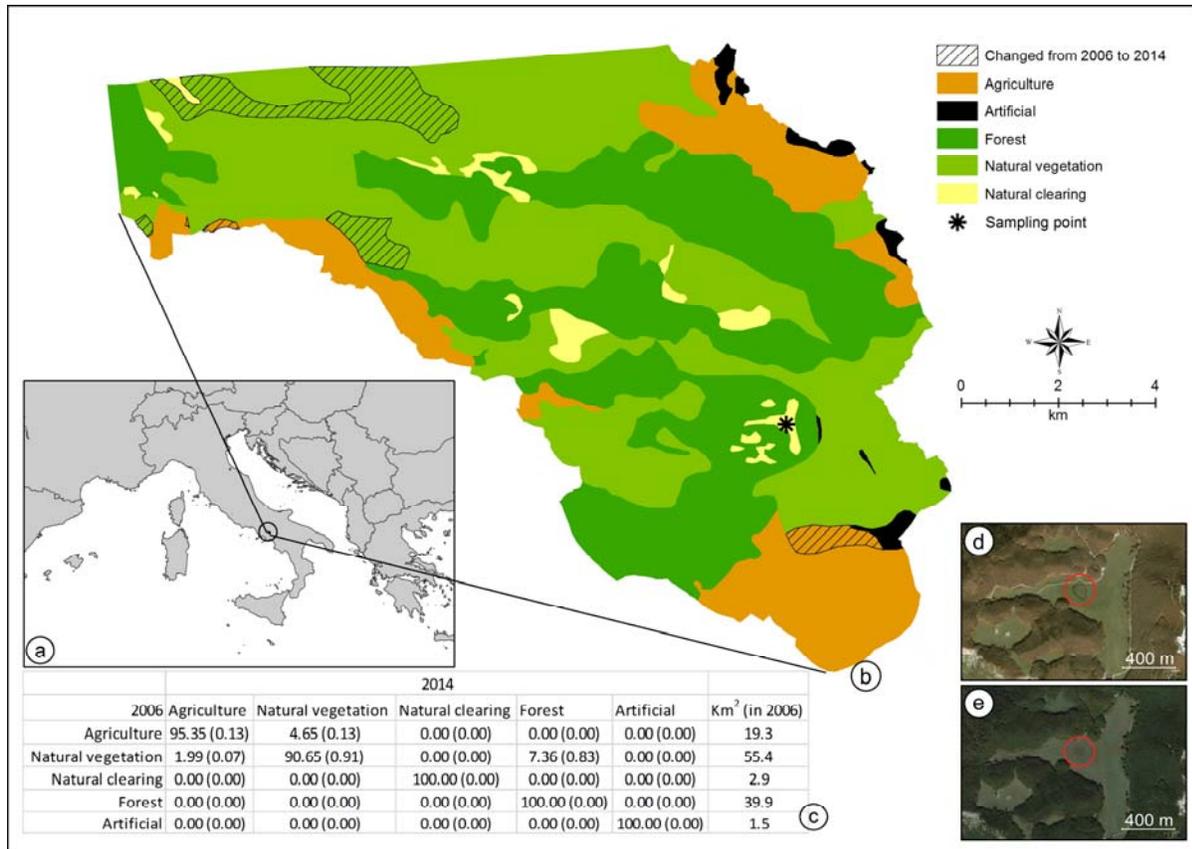
467 **Figures**

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470 **Figure 1.** Interspecific hybridisation between the common toad (*Bufo bufo*) and the Balearic
471 green toad (*Bufotes balearicus*) in the wild. The hybrid couple (a) was found spawning (b) on 10
472 May 2014, at lake Campo Maggiore, a high-elevation pond within the Partenio Regional Park,
473 southern Italy (latitude: 40.9429° N; longitude: 14.7096° E; altitude: 1330 MASL). The majority
474 of tadpoles from the hybrid egg-string reared under standard laboratory conditions were heavily
475 malformed (inset), and none survived until the metamorphosis stage; this pattern was not
476 observed for control tadpoles from co-specific mates of both species.



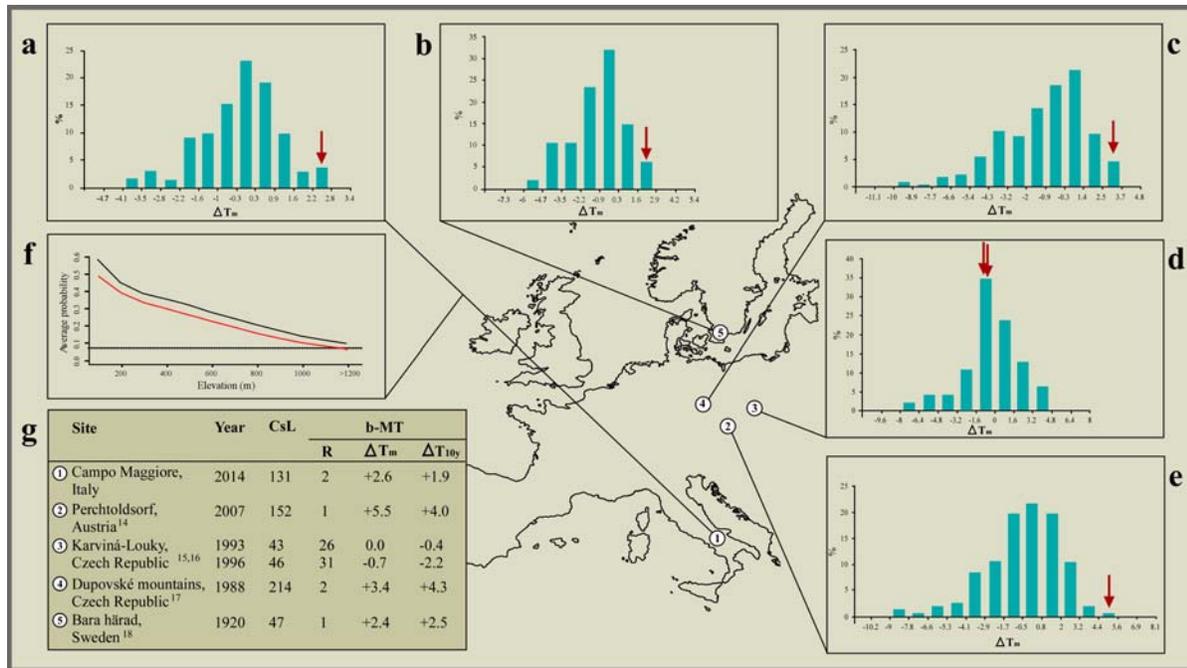
477

478 **Figure 2.** Land-use change detection analysis. (a) Location of the study area in Italy. (b) map of
 479 the land use in 2006, as obtained from direct interpretation of an aerial photo collected on 31
 480 October 2006; in the same map, the exact location where the hybridisation event has been
 481 registered is indicated, as well as all areas where land use was different when compared to a
 482 second aerial photo collected on 9 October 2014. Both aerial photos were obtained from Google
 483 Earth Pro 7.1.5.1557 (Google Inc., Mountain View, California). (c) Average percent change
 484 (range of percent change in parenthesis) in land use classes from 2006 to 2014; total km² area for
 485 each land cover class in 2006 is provided in the last column. Aerial photos of the breeding site
 486 and its neighbourhoods collected on 31 October 2006 (d), and 9 October 2014 (e).

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491 **Figure 3.** Climate correlates of the interspecific hybridisation events observed in the wild
492 between the common toad (*Bufo bufo*) and the Balearic green toad (*Bufo balearicus*). Bar
493 plots showing frequency distribution (percent) of bimonthly mean temperature deviations (ΔT_m)
494 from the 1961–1990 average, compared to the two months preceding the breeding activity at
495 each geographic region: (a) December to January, (b) February to March, and (c–e) January to
496 February. Values for the years when hybrid mates were observed are marked using red arrows.
497 Optimal bar width was computed for each climatic series following the Freedman–Diaconis rule.
498 (f) average probability of presence vs elevation at sea level (m) as modelled for the pre-2000
499 climate (red line), and for the 2007–2013 climate (solid black line); the black dotted line
500 indicates the minimum plausible level of probability of presence, above which the species can be
501 considered present, while below is considered absent. (g) Mean temperature data for each site,
502 and year of observation of interspecific mates. CsL: climatic series length, in years, before the
503 observed event; b-MT: bimonthly mean temperature; R: rank over the entire climatic series (1=
504 mildest); ΔT_m : deviation from the 1961–1990 average temperature ($^{\circ}\text{C}$); ΔT_{10y} : deviation from
505 the preceding 10-year average temperature ($^{\circ}\text{C}$).