1 **Climate change promotes hybridisation between deeply divergent**

2 **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

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

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

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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.

4

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

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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 km^2, 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

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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

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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 (Δ°C) and relative precipitation differences (% change). By using bilinear

158 resampling, we downscaled the anomalies to 0.0083° of spatial resolution (≈ 1-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 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, 199), 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.

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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   Koebenhavn Landbohojskolen, 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.

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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

Results and Discussion

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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'.

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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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457

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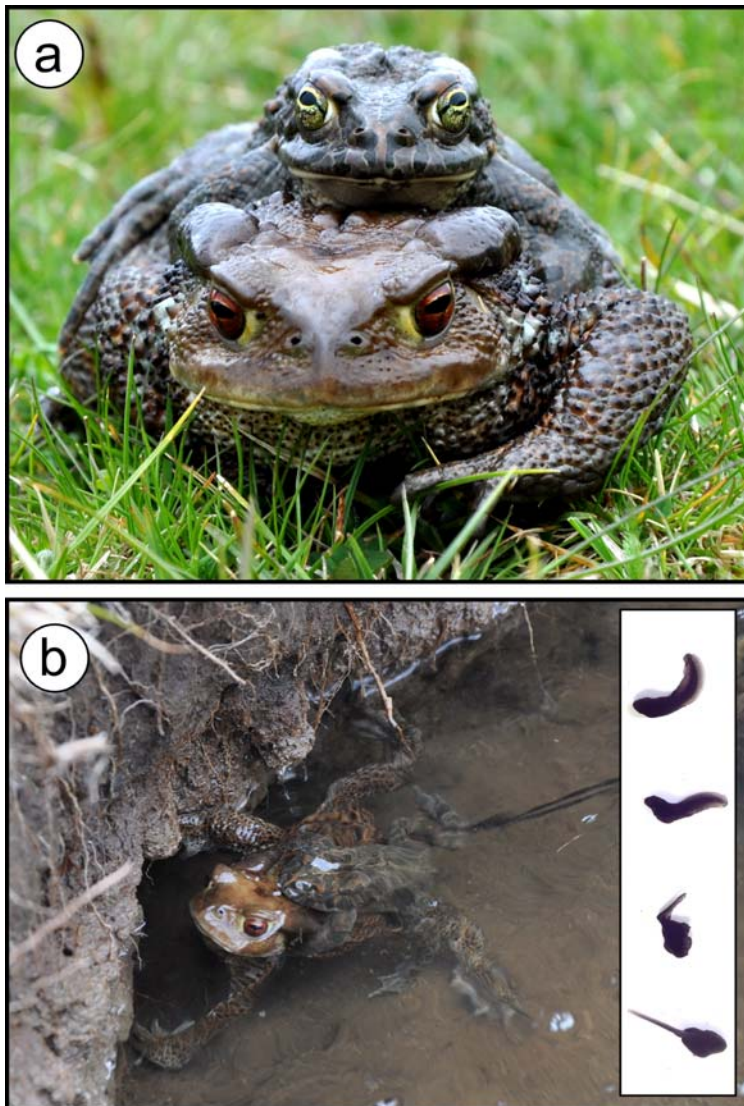462

463 **Conflict of interest**

464 The authors have no conflict of interests to declare.
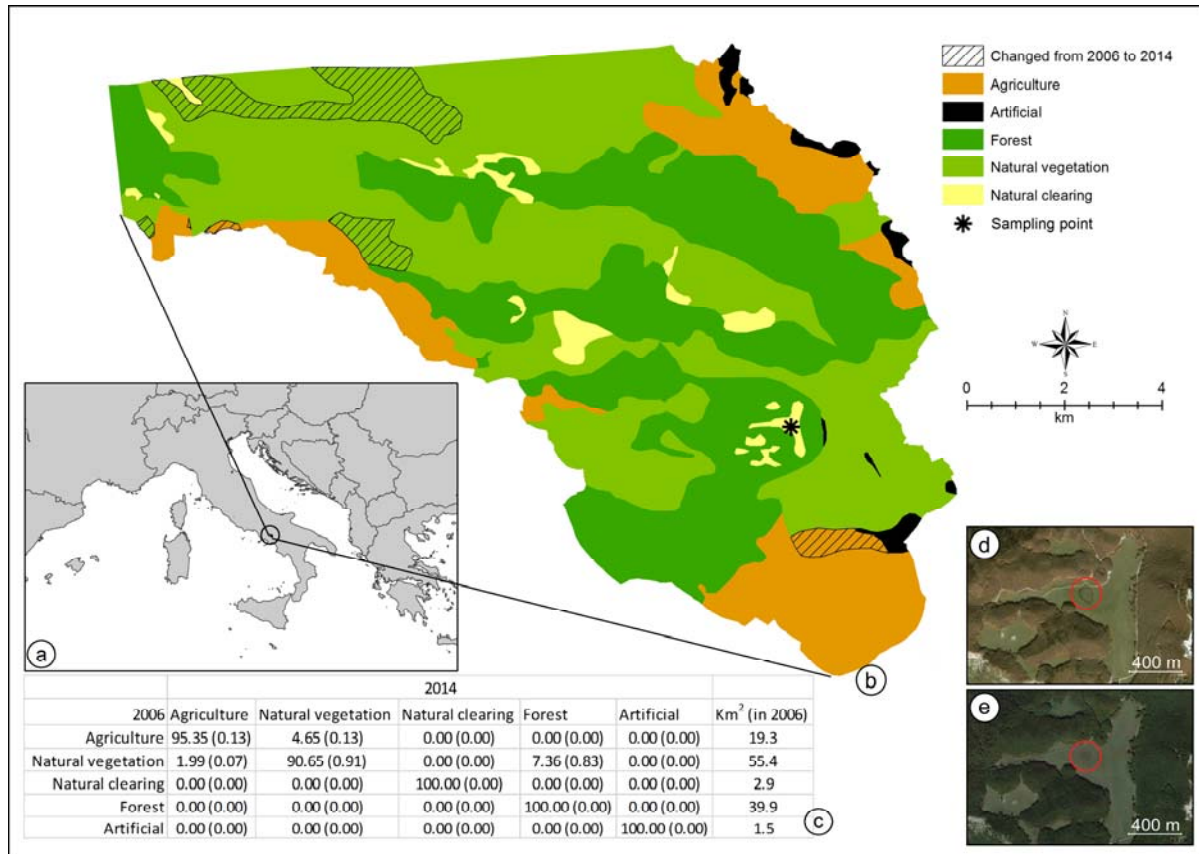
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467 **Figures**

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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.

23

	2014					
2006	Agriculture	Natural vegetation	Natural clearing	Forest	Artificial	Km² (in 2006)
Agriculture	95.35 (0.13)	4.65 (0.13)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	19.3
Natural vegetation	1.99 (0.07)	90.65 (0.91)	0.00 (0.00)	7.36 (0.83)	0.00 (0.00)	55.4
Natural clearing	0.00 (0.00)	0.00 (0.00)	100.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.9
Forest	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	100.00 (0.00)	0.00 (0.00)	39.9
Artificial	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	100.00 (0.00)	1.5

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).

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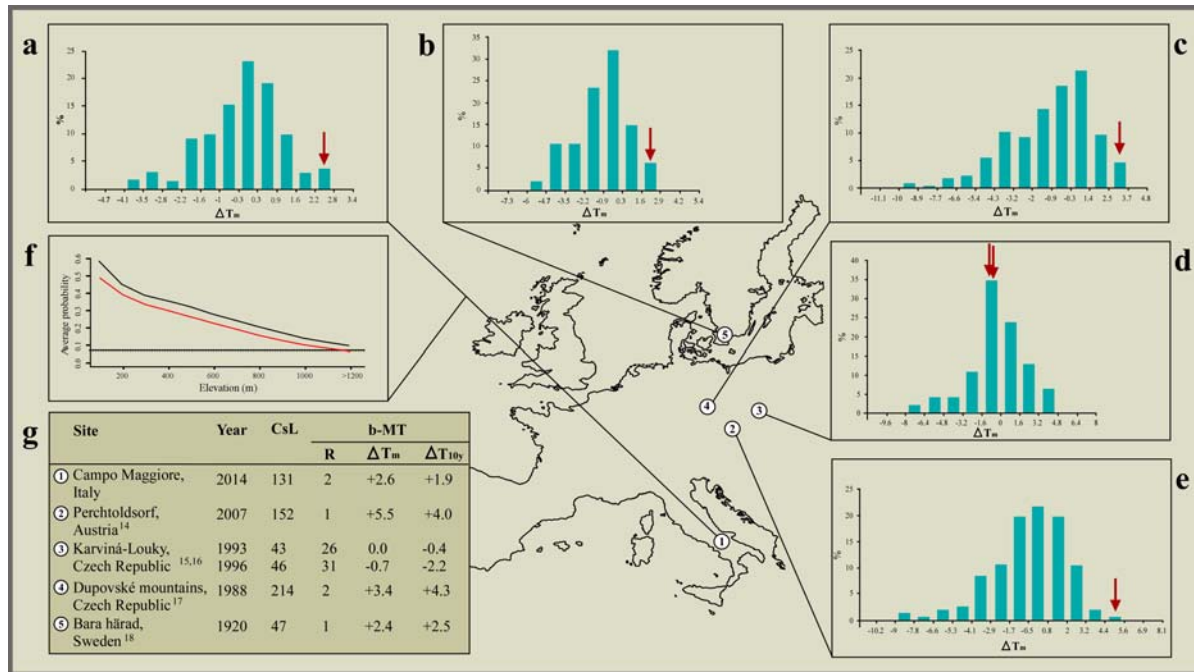
Figure 3. Climate correlates of the interspecific hybridisation events observed in the wild between the common toad (*Bufo bufo*) and the Balearic green toad (*Bufotes balearicus*). Bar plots showing frequency distribution (percent) of bimonthly mean temperature deviations (ΔTm) from the 1961–1990 average, 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).