

# Hybridization rate and climate change: are endangered species at risk?

R. A. Sánchez-Guillén · J. Muñoz · J. Hafernik ·  
M. Tierney · G. Rodríguez-Tapia · A. Córdoba-Aguilar

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**Abstract** Many species are altering their geographic range due to climate change creating new sympatric populations of otherwise allopatric populations. We investigated whether climate change will affect the distribution and thus the pattern of hybridization between two pairs of closely related damselfly species [*Ischnura damula* and *I. demorsa*, and *I. denticollis* and *I. gemina* (this, an endangered species)]. Thus, we estimated the strength of pre and postmating reproductive barriers between both pairs of species, and we predicted future potential distribution under four different Global Circulation Models and a realistic emissions scenario of climate change by using maximum entropy modelling technique. Our results showed that reproductive isolation (RI) is complete in *I. damula* × *I. demorsa* individuals: F<sub>1</sub> (first generation) hybrids are produced but do not reach sexual maturation. However, RI in *I. denticollis* × *I. gemina* hybrids is high but incomplete and unidirectional: only *I. gemina* females

produced F<sub>1</sub> hybrids which mate with males and females of *I. denticollis* and between them producing BC<sub>1</sub> (backcrosses) and F<sub>2</sub> (second generation) viable hybrids. Maximum entropy models revealed a northern and westward shift and a general reduction of the potential geographic ranges. Based on the pattern of hybridization, for *I. damula* and *I. demorsa* there is a current threat as well as a rapid displacement and/or extinction of *I. gemina* by *I. denticollis*. However, the current pattern of extinction may not continue due to the contraction in ranges of the four species.

**Keywords** Climate change · Range shifts · Sympatric distributions · MAXENT · Hybridization and introgression · Displacement and extinction

## Introduction

Currently, there is an increasing body of evidence of altered range distributions in response to rising global

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R. A. Sánchez-Guillén (✉) · A. Córdoba-Aguilar (✉)  
Departamento de Ecología Evolutiva, Instituto de Ecología,  
Universidad Nacional Autónoma de México, Apdo. Postal  
70-275, Ciudad Universitaria, 04510 Mexico, DF, Mexico  
e-mail: rguillen@uvigo.es

A. Córdoba-Aguilar  
e-mail: acordoba@ecologia.unam.mx

J. Muñoz  
Real Jardín Botánico (RJB-CSIC), Plaza de Murillo 2,  
28014 Madrid, Spain

J. Muñoz  
Centro de Biodiversidad y Cambio Climático, Universidad  
Tecnológica Indoamérica, Machala y Sabanilla, Quito, Ecuador

J. Hafernik  
Department of Biology, San Francisco State University,  
San Francisco, CA 94132, USA

M. Tierney  
USDA Forest Service, Tahoe National Forest, 15924 Hwy 49,  
Camptonville, CA 95922, USA

G. Rodríguez-Tapia  
Unidad de Geomática, Instituto de Ecología, Universidad  
Nacional Autónoma de México, Mexico, DF, Mexico

temperatures (see Parmesan et al. 1999; Parmesan 2006). It is known that insects are prone to respond more quickly than long-lived organisms to such range changes because of their short generation times and high reproductive rates (Bale et al. 2002; Gullan and Cranston 2010). For instance, in butterflies, 62 % of the studied European taxa have experienced a northward shift in geographic range (Parmesan et al. 1999), while in odonates, 39 out of 41 non-migratory British species, have shifted their ranges northwards by 62–86 km (Hickling et al. 2005).

Two consequences of climate-induced range shifts include: (a) the formation of new sympatric populations, which may favor interspecific interactions and hybridization between formerly allopatric populations of a species (Garroway et al. 2010) and, (b) the increase of sympatry degree in formerly sympatric populations, which may alter the balance between the occasional production of hybrids and their removal by natural selection (Taylor et al. 2006; Sánchez-Guillén et al. 2013). In fact, several cases of hybridizing species clearly linked to rising global temperatures have been detected in very distant taxa such as mammals [e.g. bears: *Ursus arctos* and *U. maritimus* (Kelly et al. 2010) and flying squirrels: *Glaucomys sabrinus* and *G. volans* (Melo et al. 2007)]; fish [e.g. trout: *Oncorhynchus clarki lewisi* and *O. mykiss* (Rubidge and Taylor 2004)]; and insects [e.g. damselflies *Ischnura elegans* and *I. graellsii* (Sánchez-Guillén et al. 2011a), and brown argus butterflies: *Polyommatus agestis* and *P. artaxerxes* (Mallet et al. 2011)]. Hybridization can, in turn, lead to different evolutionary outcomes. For example, introgressive hybridization can generate new genetic variation, facilitating adaptation by mixing the genomes of different species (Hoffmann and Sgrò 2011); the role of introgressive hybridization in generating adaptive wing colour diversity has been investigated in mimetic *Heliconius* butterflies (Pardo-Díaz et al. 2012). Introgression has also potential to lead to hybrid speciation (see Rhymer and Simberloff 1996) or species' extinction (Rhymer and Simberloff 1996; Allendorf et al. 2001), e.g. speciation in reverse has been detected in three-spined sticklebacks (Taylor et al. 2006), and local extinction in ischnuran damselflies (Sánchez-Guillén et al. 2011a).

Interspecific interactions and hybridization in odonates seem to be more common than previously thought (see Sánchez-Guillén et al. 2011a). In a recent paper, Sánchez-Guillén et al. (2014) did a comprehensive review of interspecific interactions and hybridization in three damselfly families, including 31 species pairs (belonging to eight different genera), and detected hybrids in 16 pairs. Partly, hybridization in these animals can be understood in the context of rising global temperatures. For example, changes in distribution in the damselfly *I. elegans* was clearly consistent with a response to increasing

temperatures in Europe (Wellenreuther et al. 2011). This expansion has led to an increase in sympatric ranges and hybridization between *I. elegans* and its sister species *I. graellsii* with an unidirectional introgression of genes of *I. graellsii* in *I. elegans* (Sánchez-Guillén et al. 2011a). The consequence was the local extinction of *I. graellsii* from areas that were colonized by *I. elegans* (Sánchez-Guillén et al. 2011a), even though the strength of pre- and post-mating reproductive barriers was almost complete (around 95 %) (Sánchez-Guillén et al. 2012). How widespread/common this trend of extinction via hybridization of extended ranges, needs to be investigated.

In the present study, we investigated: (a) the strength of reproductive isolation (RI) under laboratory conditions; (b) the pattern of hybridization in natural sympatric populations; and (c) potential direct (over parental species) and indirect (over offspring) costs of interspecific interactions and hybridization assuming hypothetical scenarios of increased sympatry due to rising global temperatures. For this, we used two pairs of *Ischnura* damselfly species: *I. damula* and *I. demorsa*, and *I. denticollis* and *I. gemina*, which are sympatric in some populations. First, we measured the strength of total RI and the contribution of each individual barrier to total RI in *I. damula* × *I. demorsa*, and in *I. denticollis* × *I. gemina*. This was done by using data from previous studies by Tierney (1996) and Johnson (1975). Second, we estimated their potential current and potential future distributions and predicted future potential overlapping ranges, using the maximum entropy modeling technique (MAXENT) as it is one of the best modeling methods available (Elith et al. 2006; Elith and Leathwick 2009). Our first step provides the necessary information to visualize not only patterns of RI, but also patterns of hybridization and/or introgression. The second step allows us to put forward a potential threatening scenario for those species that may face extinction given an increased rate of hybridization and/or introgression. Notice that we make use of a rare species (*I. gemina*), which is currently red listed by the World Conservation Union as Endangered (Abbott 2007).

## Materials and methods

### Species' relevant biology and distribution

The damselflies *I. damula* and *I. demorsa* are genetically and morphologically similar (Chippindale et al. 1999). They are distributed in North America and are mainly allopatric (Chippindale et al. 1999), but sympatric in 22.27 % of the *I. demorsa* and 30.16 % of *I. damula* localities used in this study. In sympatry, hybridization is common with males and females of both species engaging

in heterospecific matings (Johnson 1975), but without introgression of genes, because hybrids are not viable (Johnson 1975). On the other hand, *I. denticollis* and *I. gemina* are genetically different (Chippindale et al. 1999) but morphologically similar (Tierney 1996; Moore 2007) although they can be unambiguously distinguished by their anal appendage morphology (Leong and Hafernik 1992). *Ichnura denticollis* is widely distributed in Mexico and the southwestern United States (Tierney 1996). Conversely, *I. gemina* has one of the most restricted odonate distributions, occurring only in the San Francisco Bay Area (Garrison and Hafernik 1981), where six populations (31.57 % of the *I. gemina* localities used here) are sympatric with *I. denticollis*. Partly, due to its restricted distribution, *I. gemina* was the object of a reintroduction program (Hannon and Hafernik 2007). Recently, *I. denticollis* has increased its distribution overlapping with *I. gemina* populations in the San Francisco Bay Area as both species occupy similar habitats (Leong and Hafernik 1992) but currently, most of the sympatric areas for *I. gemina* and *I. denticollis* seem to support only *I. denticollis*. *Ichnura gemina* appears absent from most of the locations south of San Francisco where it was found in the 80s and 90s (personal observation). In sympatry, *I. gemina* females are involved in heterospecific matings, while females of *I. denticollis* avoid heterospecific matings with males of *I. gemina* due to mechanical incompatibility. Additionally, matings between *I. gemina* females and *I. denticollis* males form hybrids. This pattern of hybridization leads to a unidirectional introgression of genes from *I. gemina* to *I. denticollis* (Leong and Hafernik 1992; Tierney 1996; Moore 2007).

#### Strength of reproductive isolation

We compiled data from Johnson (1975) and Tierney (1996) consisting of 24 crosses of *I. damula* × *I. demorsa*, and 26 crosses of *I. denticollis* × *I. gemina*, respectively. Johnson (1975) sampled *I. damula* and *I. demorsa* from 14 sympatric localities in southwestern New Mexico from 1962 to 1967. From these samples, we estimated proportions of conspecific and heterospecific matings: 102 conspecific matings of *I. damula*, 123 conspecific matings of *I. demorsa*, 18 heterospecific matings between *I. damula* males and *I. demorsa* females and 17 heterospecific matings between *I. demorsa* males and *I. damula* females. Additionally, Johnson (1975) transported females in heterospecific tandem post-copula ( $n = 8$  for *I. damula*, and  $n = 10$  for *I. demorsa*), and in conspecific tandem post-copula ( $n = 4$  for *I. damula*, and  $n = 2$  for *I. demorsa*) to the laboratory to obtain eggs. These females were allowed to oviposit in filter papers which were maintained in petri dishes with water until egg hatching (details in Johnson 1975). During 1985, *I. denticollis* and *I. gemina* were

sampled from four allopatric localities: Monterey, Merced and Alameda counties (*I. denticollis*); and San Mateo (*I. gemina*) (Hafernik 1988). Adults were maintained in separate cages and were fed with *Drosophila*. Humidity within the cages was maintained by means of pint plastic deli containers filled with water and the temperature ranged from 15 to 30 °C. After mating (conspecific and heterospecific) females were allowed to lay eggs in filter papers, which were placed in petri dishes and covered with water until egg hatching. Larvae were fed with *Artemia salina* nauplii and maintained in aquaria until adult emergence (see details in Tierney 1996).

In order to estimate total strength of RI, we used the reproductive categories proposed by Sánchez-Guillén et al. (2012). Thus, we estimated three pre-mating barriers (sexual, mechanical<sub>I</sub> and mechanical<sub>II</sub>) (details in Supplemental Tables S1, S2), three post-mating, prezygotic, barriers (oviposition, fecundity and fertility) and five post-mating, postzygotic, barriers (F<sub>1</sub>-survivorship, F<sub>1</sub>-sex-ratio, F<sub>1</sub>-mechanical<sub>I</sub>, F<sub>2</sub>-oviposition and F<sub>2</sub>-survivorship) (details in Supplemental Table S3). Calculation details are summarized in Table 1.

We used the multiplicative function of individual components of isolation in sequential mating stages to estimate the cumulative RI in *I. damula* × *I. demorsa*, and in *I. denticollis* × *I. gemina* (Coyne and Orr 1989, 1997; Ramsey et al. 2003). This method allows the quantification of the contribution of each individual barrier to total RI. The absolute contribution (AC) of a component to RI at stage  $n$  was calculated using the following equation, after Ramsey et al. (2003):

$$AC_n = \left( 1 - \sum_{i=1}^{n-1} AC_i \right)$$

The AC of sexual isolation equals  $AC_1 = RI_1$  because it acts first. However, the AC of the second acting barrier, mechanical<sub>I</sub> isolation ( $AC_2$ ) equals  $RI_2 (1 - AC_1)$ , and mechanical<sub>II</sub> isolation ( $AC_3$ ) equals  $RI_3 [(1 - (AC_1 + AC_2))]$ , and so on. Total RI is the sum of the ACs of sexual, mechanical<sub>I</sub>, mechanical<sub>II</sub>, oviposition, fecundity, fertility, F<sub>1</sub>-survivorship, F<sub>1</sub>-sex-ratio, F<sub>1</sub>-mechanical<sub>I</sub>, F<sub>2</sub>-oviposition and F<sub>2</sub>-survivorship barriers.

#### Potential current and future distribution

Our study area included Mexico and the southwestern United States covering land between the latitudes 40.00–10.00°N, and the longitudes 130.00–90.00°W. As bioclimatic variables we used the WorldClim 1.4 ([www.worldclim.org](http://www.worldclim.org)) data set (Hijmans et al. 2005) at 0.008333° cell size. To establish a set of uncorrelated climatic variables, we intersected the variables with 10,000 points

**Table 1** Estimates of the strength of the reproductive isolation index ‘S’ for each isolating barrier

Barrier	Isolation measure	Range	Formula	♂DEN-♀GE		♂GE-♀DEN		♂DA-♀DEM		♂DEM-♀DA	
				S	AC	S	AC	S	AC	S	AC
Premating	Sexual	-1 to 1	$RI_{\text{sexual-mechanical}} = 1 - (\text{observed heterospecific attempts to tandem under random mating}) / \text{heterospecific mating expected}$	0.5156	0.5156	0.2361	0.2361				
	Mechanical <sub>I</sub>	0 to 1	$RI_{\text{mechanical I}} = 1 - (\text{tandem attempts/tandems})$	0.9954	0.4822	0.7637	0.5834				
	Mechanical <sub>II</sub>	0 to 1	$RI_{\text{mechanical II}} = 1 - (\text{tandems/matings})$	0.3863	0.0009	0.7552	0.1363	0.7000	0.7000	0.7570	0.7570
	Oviposition	-1 to 1	$RI_{\text{sexual-mechanical}} = 1 - (\text{observed heterospecific matings under random mating}) / \text{heterospecific mating expected}$	0.0000	0.0000	0.0000	0.0000	0.3000	0.0900	0.4000	0.0972
Postmating, prezygotic	Fecundity	-1 to 1	$RI_{\text{oviposition}} = 1 - (\% \text{ females oviposited heterospecific matings} / \% \text{ females oviposited conspecific matings})$	0.6945	0.0010	na	na	0.7235	0.1519	0.5932	0.0865
	Fertility	-1 to 1	$RI_{\text{fecundity}} = 1 - (\text{mean fecundity heterospecific matings} / \text{mean fecundity conspecific matings})$	0.3021	0.0001	-0.1482	-0.0066	0.5721	0.0332	0.3803	0.0226
Postmating, postzygotic	F <sub>1</sub> -survivorship	-1 to 1	$RI_{\text{fertility}} = 1 - (\text{mean fertility heterospecific matings} / \text{mean fertility conspecific matings})$	1.0000	0.0003	0.3636	0.0184	1.000	0.0248	1.000	0.0368
	F <sub>1</sub> -sex-ratio	-1 to 1	$RI_{F_1\text{-survivorship}} = 1 - (\text{fitness hybrid} / \text{fitness parent species})$	-	-	-0.0933	-0.0030	-	-	-	-
	F <sub>2</sub> -mechanical	-1 to 1	$RI_{F_1\text{-sex-ratio}} = 1 - (\text{fitness hybrid} / \text{fitness parent species})$	-	-	-0.3277	-0.0116	-	-	-	-
	F <sub>2</sub> -oviposition	-1 to 1	$RI_{F_2\text{-mechanical}} = 1 - (\text{fitness hybrid} / \text{fitness parent species})$	-	-	0.4372	0.0205	-	-	-	-
Total pre- and post-zygotic isolation	F <sub>2</sub> -survivorship	-1 to 1	$RI_{F_2\text{-oviposition}} = 1 - (\text{fitness hybrid} / \text{fitness parent species})$	-	-	-0.1415	-0.0037	-	-	-	-
				1.0000	0.9690	1.0000	1.0000				

Contributions to total reproductive isolation were calculated for sequential reproductive barriers, with the sum of contributions equalling total isolation. ‘S’ denotes the relative strength and ‘AC’ the absolute contribution of different pre- and postzygotic isolating barriers in reciprocal crosses between *I. denticollis* and *I. gemina* (♂DEN-♀GE: *I. denticollis* male and *I. gemina* female, ♂GE-♀DEN: *I. gemina* male and *I. denticollis* female) and between *I. damula* and *I. demorsa* (♂DA-♀DEM: *I. damula* male and *I. demorsa* female, ♂DEM-♀DA: *I. demorsa* male and *I. damula* female). ‘na’ denotes cases where the isolation index could not be estimated, and ‘-’ cases were the isolation index could not be estimated because the strength of the previous barrier was complete. Total reproductive isolation for a set of components is the sum of all the AC

randomly selected in the extension of the study area, ran an exploratory data analysis and a correlation analysis, and eliminated one of the variables in each pair with a Pearson correlation value  $>0.7$ . The final data set includes annual mean temperature (bio 01), max temperature of warmest month (bio 05), temperature annual range (bio 07), annual precipitation (bio 12), precipitation seasonality (bio 15), precipitation of warmest quarter (bio 18), precipitation of coldest quarter (bio 19).

Presences of the four species were compiled from specimens kept at the Essig Museum of Entomology (University of California at Berkeley), literature records (Tierney 1996; Moore 2007), confirmed records in Odonata Central (<http://www.odonatacentral.org>; accessed 1-July-2013), and GBIF records carefully checked for geographic accuracy ([www.gbif.org](http://www.gbif.org); accessed 17-July-2013). Some records are registered to county, which are rather heterogeneous in size, ranging from small and climatically homogeneous in the US Great Plains, to large and heterogeneous at the US Pacific coast; to avoid inaccurate georeferentation, only county records from the Great Plain distributions were accepted. The final data set includes 253 unique presences for *I. damula* (at  $0.008333^\circ$  pixel size), 185 for *I. demorsa*, 408 for *I. denticollis*, and 19 for *I. gemina*.

In odonates, distribution is mainly affected by eco-physiological traits (Corbet and May 2008). Thus, in order to predict future overlapping geographic ranges between *I. denticollis* and *I. gemina*, and between *I. damula* and *I. demorsa*, we simulated the areas with future climatic conditions equivalent to those of its present range. This does not mean that the species will occupy all these areas, as we did not take into account population dynamics or dispersal abilities (see Huntley et al. 2008 for similar trends). Nevertheless, these areas will experience climatic conditions equivalent to the climatic conditions of currently occupied areas.

Species distribution models were generated with Maxent 3.3.3k (Phillips et al. 2006). Models were constructed setting several parameters to default ('Auto features', convergence =  $10^{-5}$ , maximum number of iterations = 500, background = 10,000) and varying the prevalence (0.5, 0.6 and 0.7) and regularization value  $\beta$  (1, 2 and 3) to find which combination of settings and variables generated the best outcomes (highest area under the curve, or AUC) while minimizing the number of model parameters, as well as producing 'closed', bell-shaped response curves guaranteeing model transferability (Elith et al. 2010). A regularization multiplier higher than 1.0 allows that variables' average values in the projections spread from the empirical average of the background points (the situation if it is set to 1.0), avoiding model overfitting (Warren and Seifert 2011), and smoothing the response

curves. In total, 27 models were generated for each species (3 variables schemes  $\times$  3 regularization values  $\times$  3 prevalence schemes). Performance of the models was assessed by means of the AUC in a ROC statistic through tenfold cross-validation, and minimizing the number of model parameters.

Continuous Maxent outcomes of the best models were transformed to presence/absence models using the '10 percentile training presence', and projected to three future time slices: 2020, 2050 and 2080. We generated projections for the four GCM with data available at the CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS) spatially downscaled using the Delta Method (<http://www.ccafs-climate.org>): CCCMA-CGCM2, CSIRO-MK2.0, UKMO-HADCM3, and MIROC-NIESS99) and the A2a emissions scenario (IPCC 2007). The final presence/absence model for each species and time slice was the area where the four models predicted presence for the species considered (red areas in Figs. S1–S4). To confirm that combinations of novel climates were not a cause of concern in the projections, we generated the multivariate environmental similarity surfaces (MESS, see Elith et al. 2010); this grid was reclassified and values below zero were masked to show areas of novel climate space relative to the range under which the model was fitted (gray to black areas in Figs. S1–S4).

Using this consensus models, we estimated the expansion or contraction of the distribution ranges as the proportion in  $\text{km}^2$  that each species will occupy in each future time slice with regard to its own potential current distribution or, for overlapping ranges, with regard to the species being compared.

## Results

### Strength of reproductive isolation

#### *Premating barriers: sexual and mechanical isolation*

Sexual and mechanical isolation in *I. damula*  $\times$  *I. demorsa* were tested simultaneously in the field checking the number of conspecific and heterospecific matings (details in Supplemental Tables S1, S2). Males of *I. damula* and *I. demorsa* were involved in heterospecific matings in similar proportions, and in both cases, less often than in conspecific matings (details in Supplemental Tables S1, S2).

Sexual and mechanical isolation in *I. denticollis*  $\times$  *I. gemina* were tested under laboratory conditions (in terms of both attempting to tandem and compatibility during tandem and mating) at similar species' proportions. In both species, males attempted to mate less often with heterospecific rather than conspecific females and also achieved

fewer heterospecific matings than conspecifics because of mechanical incompatibility during tandem and mating (details in Supplemental Tables S1, S2).

*Postmating prezygotic barriers: isolation by oviposition failure, and reduced fecundity and fertility*

*Ischnura damula* and *I. demorsa* females showed reduced oviposition rates after heterospecific matings, while *I. denticollis* and *I. gemina* females involved in both conspecific and heterospecific matings did not show failure in oviposition (details in Supplemental Table S3). Fecundity (measured as the number of eggs laid in the first three clutches) and fertility (number of developed eggs) were reduced in the four heterospecific combinations (details in Supplemental Table S1).

*Postmating postzygotic barrier: hybrid inviability, hybrid sterility and breakdown of hybrid vigour*

Hybrid inviability (in terms of F<sub>1</sub>- survivorship until adulthood) was complete in all species combinations except between *I. gemina* females × *I. denticollis* males (details in Supplemental Table S3). Hybrid sterility and hybrid vigour were examined (in terms of F<sub>2</sub> mechanical isolation, F<sub>2</sub> oviposition and F<sub>2</sub> survivorship) by conducting hybrid mating experiments between F<sub>1</sub>-offspring-derived from crosses between *I. gemina* males × *I. denticollis* females with males and females of *I. demorsa* × *I. damula*. Hybrid sterility was detected, but not reduced hybrid vigour (details in Supplemental Table S3).

*Total reproductive isolation*

Total premating isolation between males of *I. denticollis* and females of *I. gemina* was high (0.9987) and similar to total premating isolation between *I. gemina* males and *I. denticollis* females (0.9558) (Table 1; Fig. 1). However, postmating barriers were less important in preventing isolation: between *I. denticollis* males and *I. gemina* females postmating prezygotic (0.0011) and postmating postzygotic (0.0003) were similar to postmating prezygotic (−0.0066) and postmating postzygotic (0.0206) that were detected between *I. gemina* males and *I. denticollis* males (Table 1; Fig. 1). Between *I. denticollis* males and *I. gemina* females, RI was complete before F<sub>1</sub> hybrids achieve sexual maturity, while between *I. gemina* males and *I. denticollis* females, RI is not complete and allows actually that F<sub>1</sub> and F<sub>2</sub> hybrids achieve sexual maturity.

Between *I. demorsa* and *I. damula* total premating isolation was high and symmetric between *I. damula* males and *I. demorsa* females (0.7000) and between *I. demorsa* males and *I. damula* females (0.7570) (Table 1; Fig. 1).

Postmating isolation was less important and also very symmetric in both crossing directions: between *I. damula* and *I. demorsa* postmating, prezygotic was 0.2752 and postmating postzygotic was 0.0225, and between *I. demorsa* and *I. damula*, postmating prezygotic was 0.2062 and postmating postzygotic was 0.0368 (Table 1; Fig. 1). In both crossing directions F<sub>1</sub> hybrids do not achieve sexual maturity.

*Predicted current and future distribution modeling*

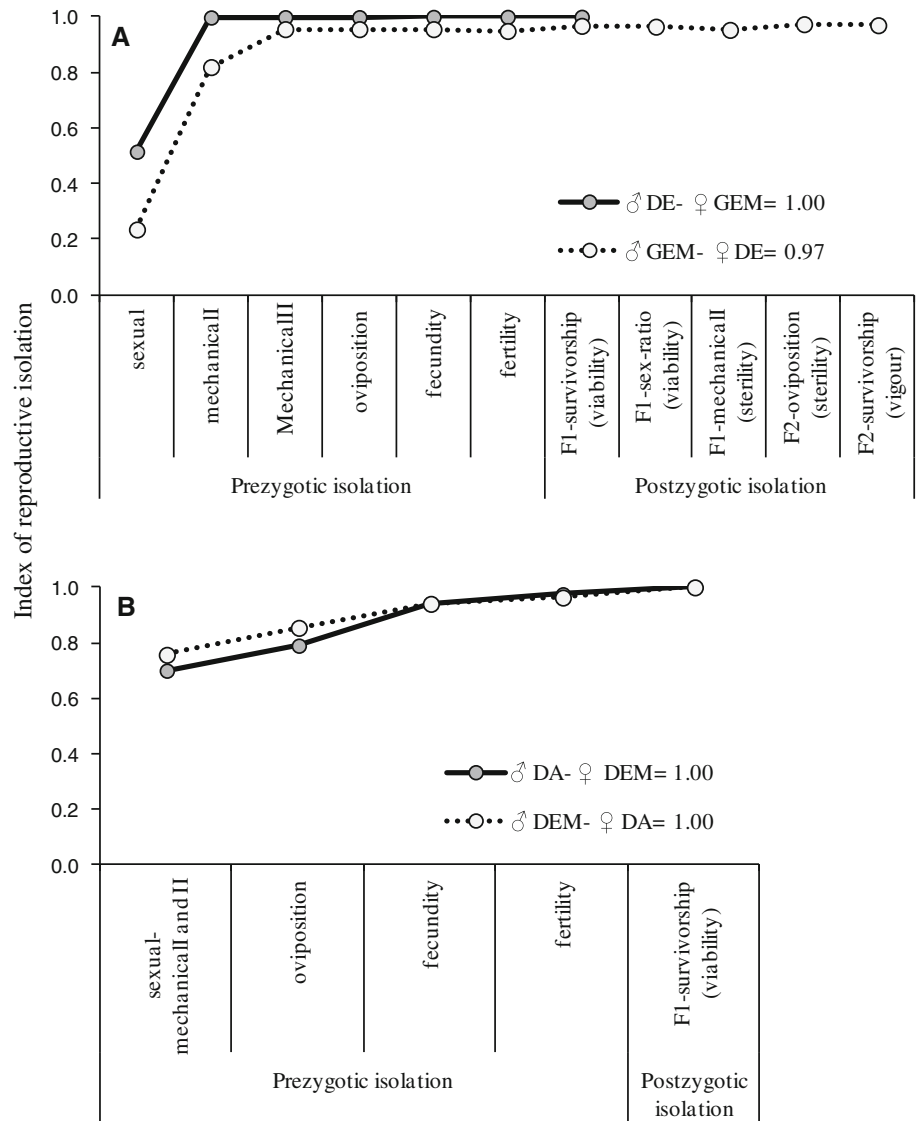
The area under the receiver operating curve (AUC) of the models ranged from 0.866 ± 0.026 (mean ± SD) of *I. damula* to 0.903 ± 0.022 of *I. demorsa*, 0.837 ± 0.029 of *I. denticollis*, and 0.998 ± 0.001 of *I. gemina*. Current predicted distributions for the four taxa indicated areas of suitable habitat which are not occupied (Fig. 2). Distribution models projected to scenario A2a indicated a general contraction of the potential ranges (Fig. 3) for the four species. For *I. damula* and *I. demorsa* the contraction will range from 21.77 to 67.95 %, while for *I. denticollis* will range from 5.67 to 10.63 % in the three time periods (2020, 2050 and 2080). Additionally, for *I. gemina* there will be an expansion of the potential range for 2020 (17.40 %) and 2050 (24.32 %), while for 2080 is predicted a contraction of 26.37 %. In consequence, pairwise overlapping distribution ranges will change. *I. damula* and *I. demorsa*, and *I. denticollis* and *I. gemina* will reduce their overlapping ranges (Table 2). Moreover, future distributional models indicated a shift of the suitable areas toward northern latitudes and western longitudes for all species except for *I. gemina* (see Supplemental Figs. S1–S4).

## Discussion

Consistent with the trend of range shift to northern regions of several African and European odonates (Hickling et al. 2005; Ott 2010; Sánchez-Guillén et al. 2014), our projections predicted a general contraction of the potential ranges, and a northern and westward shift for all species, except *I. gemina*.

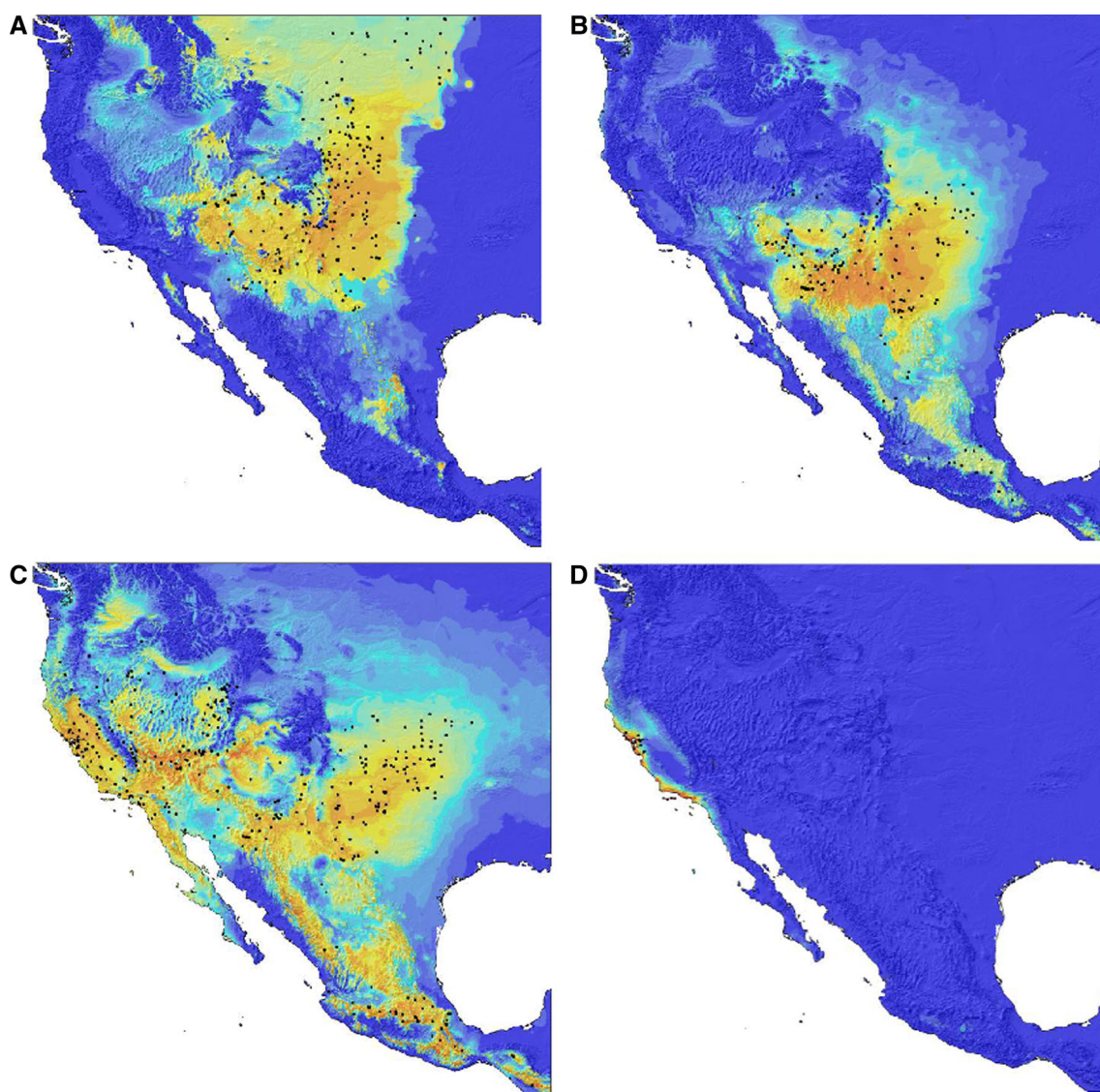
However, before discussing in detail the consequences of predicted distribution changes, it is important to understand the nature of hybridization in our study species. Hybrid formation in *I. damula* × *I. demorsa* occur in both reciprocal crossing directions, but F<sub>1</sub> hybrids do not reach sexual maturity (i.e. RI = 100 %; Fig. 1), which gives rise to a pattern of bidirectional hybridization without introgression. Conversely, in *I. denticollis* × *I. gemina*, RI is high but incomplete. *I. denticollis* males and *I. gemina* females produce F<sub>1</sub> hybrids, but these hybrids do not reach sexual maturity (RI = 100 %; Fig. 1), while *I. gemina*

**Fig. 1** Relative contributions to total isolation in reciprocal crossings following the method described by Ramsey et al. (2003). **a** *I. denticollis* × *I. gemina*, **b** *I. damula* × *I. demorsa*



males and *I. denticollis* females produce partially fertile F<sub>1</sub> hybrids (RI = 96.9 %; Fig. 1), which can mate among them and with *I. denticollis* males and females, leading to a pattern of unidirectional introgressive hybridization of genes of *I. gemina* in *I. denticollis*. This pattern of hybridization explains the presence of F<sub>1</sub> hybrids and successive backcrosses with *I. denticollis* detected in natural populations by using AFLPS markers (see Moore 2007). The presence of genes of *I. gemina* in currently allopatric *I. denticollis* populations may be indicative of past, but recent hybridization in which *I. gemina* has been displaced by *I. denticollis* by a process of unidirectional hybridization. One consequence of the past and the current displacement of *I. gemina* over *I. denticollis* populations, is the local extinction of *I. gemina*. Additionally, the introgression of genes of *I. gemina* in *I. denticollis* may have favored *I. denticollis* adaptation to new environmental

conditions (see Hoffmann and Sgrò 2011), as it has been detected in other *I. gemina* populations (Moore 2007). Interestingly, a similar process has been found in two genetically related Mediterranean damselflies, *I. elegans* and *I. graellsii* (see Sánchez-Guillén et al. 2014). Between *I. elegans*, a recent colonizer in Spain (Monetti et al. 2002; Sánchez-Guillén et al. 2005), and *I. graellsii*, an endemic species of the Iberian Peninsula, the recent range expansion of *I. elegans* has led to the displacement of *I. graellsii* by extensive introgressive hybridization of *I. graellsii* genes into *I. elegans* towards the south in the Iberian Peninsula (Sánchez-Guillén et al. 2011b; Wellenreuther et al. 2011). This displacement of the endemic species, *I. graellsii*, has taken place by extensive introgressive hybridization of *I. graellsii* genes into *I. elegans* (Sánchez-Guillén et al. 2011a). This may have favored adaptation of *I. elegans* to its new environmental conditions.



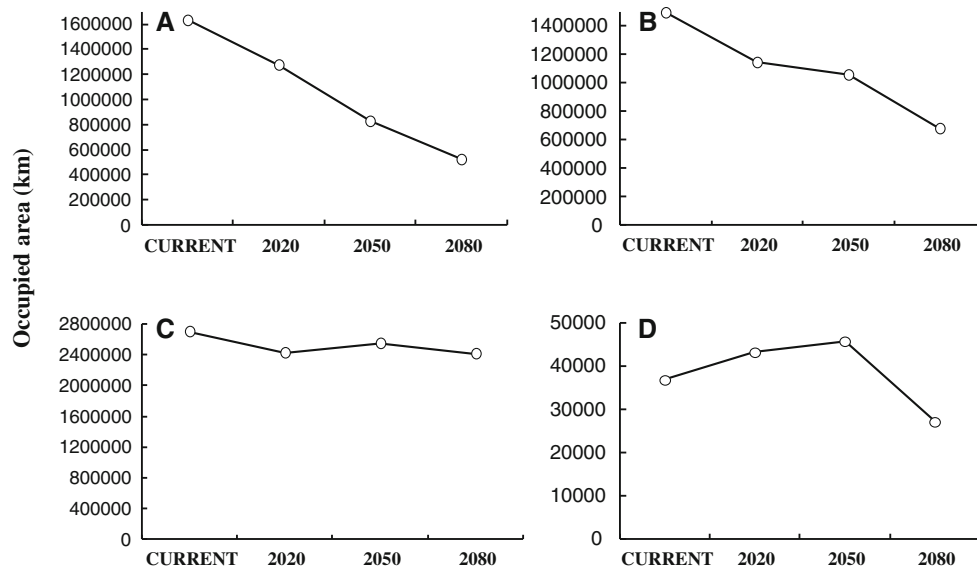
**Fig. 2** Suitability map for current climatic conditions and presences used to generate the models. **a** *I. damula*, **b** *I. demorsa*, **c** *I. denticollis*, **d** *I. gemina*. Suitability increases from dark blue (0) to green (0.5) to red (1). (Color figure online)

Despite what has occurred in the recent history of our four study species, our future predictions indicate a reduction in degree of overlapping. This contraction of ranges will lead each species to restrict its interspecific interactions with its current pair. This contraction will be especially risky for *I. gemina* given its already small distribution area. It is unclear, however, what will happen with the adapted introgressed individuals. Actually, worth studying is to know whether there will be some other interspecific interactions with species not treated in our study but that may also suffer of changes leading to communities different to those occurring now. In fact, one exercise is to use the distribution data of current odonate communities to see how much they will change in future years and predict which species pairs, according to genetic

distance (Sánchez-Guillén et al. 2014), will be more likely to produce hybrids. For example, there are at least 13 ischnuran species in North America which, in many cases, currently overlap in range according to Odonata Central (<http://www.odonatacentral.org>). How much their distribution will change producing new combinations of potentially hybridizing species including the ones we studied here, we do not know. Clearly this is an area of further research.

Long-term evolutionary effects of hybridization on species and populations are of special concern when they involve endangered species or disturbed landscapes (Beaumont et al. 2001), mainly given the implications for legal protection (Rhymer and Simberloff 1996; Beaumont et al. 2001). When hybridization of an endangered species





**Fig. 3** Predicted current binary (presence/absence) distribution and predicted distribution for three time periods (2020, 2050 and 2080) under IPCC scenario A2a for the four species. *I. damula* (a), *I. demorsa* (b), *I. denticollis* (c), and *I. gemina* (d)

**Table 2** Percentage of overlapping areas between each future projection (2020, 2050 and 2080) under scenario A2a and the actual potential occupied range

Overlapping species	Overlapped species	Potential actual	Potential 2020	Potential 2050	Potential 2080
<i>I. damula</i>	<i>I. demorsa</i>	52.40	48.09	23.85	12.63
<i>I. demorsa</i>	<i>I. damula</i>	47.96	43.11	30.46	16.42
<i>I. denticollis</i>	<i>I. gemina</i>	99.81	78.16	72.99	61.19
<i>I. gemina</i>	<i>I. denticollis</i>	1.36	1.40	1.31	0.69

is discovered, management actions need to consider the effects to a species’ range with respect to the potential to change hybridization rates (Wolf et al. 2001). This is indeed the case for *I. denticollis*, given its extensive distribution (Tierney 1996) and population density (e.g. Córdoba Aguilar 1993). Thus, although efforts have been directed to protect *I. gemina* via reintroduction (Hannon and Hafernik 2007), further measures should be taken at least currently before it is too late and present-day *I. gemina* disappears. Conservation biologists should think carefully about the more effective strategies to protect this animal.

In conclusion, our study reveals a current threat to common and rare species via hybridization between closely related species induced by range expansions. This risk, however, cannot necessarily be extrapolated to future scenario of global change although we are just considering two species pairs.

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