**Interspecific hybridization in insects in times of climate change**

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**Introduction**

Insects are one of the most abundant group of living beings, comprising 54% of all known species and the most spread all over the planet, inhabiting terrestrial and aquatic ecosystems (Gullan & Cranston, 2010). Insects’ distribution is strongly affected by temperature, especially winter temperature (Bale & Hayward, 2010). This is not only because of their development, reproduction and survival are strongly influenced by temperature and precipitation but also because of their short generation times and high reproductive rates (Bale *et al.*, 2002). Musolin et al. (Musolin & Saulich, 2012) point out that insect responses to global warming (changes in ranges, abundance, phenology, voltinism, morphology, physiology, behavior and species interactions) are likely species-specific (or even population-specific). In that way, insects respond quickly to climate change with three main responses: migration, adaptation and extinction (Menéndez, 2007). Migration allows insect populations follow favorable conditions while adaptation allows insects populations survive in newly altered environments. However, not all species will be able to move or adapt quickly enough, thus they could be locally or completely extinct.

Insect’s distribution is changing in unprecedented ways, in form of expansion (fast growing, multivoltine and non-diapausing species) and contraction (slow growing species which need low temperatures to induce diapause) of their geographic and altitudinal ranges (Bale *et al.*, 2002; Menéndez, 2007). Evidence of poleward shifts and expansions come from different insect orders with high mobility such as Lepidopterans, Heteropterans Neuropterans, Orthopterans and Odonates (Sánchez-Guillén *et al.*, 2016). Climate-induced range shifts can increase the potential of hybridization by forming new sympatric distributions between species that have diverged in allopatry and have not completed their reproductive isolation thus forming new hybrid zones or increasing the sympatry between parapatric or partially sympatric species, altering the stablished equilibrium in the hybrid zone [6,7]. Nonetheless, so far, there are scarce studies investigating hybridization in insect orders experiencing climate-induced range expansions (e.g., insect pest species (Mallet, 2018)). Current studies mainly include Odonates, Lepidopterans and Hymenopterans (reviewed in (Sánchez-Guillén *et al.*, 2016)).

The aim of this review is to synthesize most common hybridization outcomes increasing or decreasing genetic diversity and/or biodiversity during climate-induced range shifts, focusing on empirical evidence from insects. To this end we did a literature review on …

**Outcomes of climate-induced hybridization**

Most common scenarios of hybridization during climate-induced range shifts are compelled in Figure 1. When one species come into the distribution of a closely related species with which has evolved in allopatry, they can hybridize and produce hybrids if reproductive isolation is incomplete (Figure 1A-B). Hybridization outcomes will vary from increasing to reducing genetic divergence and biodiversity, and this variation depends on both, intrinsic factors such as reproductive isolation, and extrinsic factors such as environmentally dependent hybrid fitness (Pickup *et al.*, 2019). Most common scenarios increasing genetic divergence are introgression (transfer of adaptations), reinforcement of reproductive isolation, and the origin of new hybrid lineages. Moreover, hybridization can also decrease genetic divergence by the breakdown of reproductive barriers, species fusion and species extinction. When species produce hybrids, and those hybrids can only mate with one parental (unidirectional) or both parental (bidirectional) species, introgression i.e., the transfer of genetic material from one species into the gene pool of the other species, happen increasing genetic divergence and transferring adaptations (Arnold, 2004) (Figure 1C). However, when hybrids are less viable than the parental species, they are selected against by natural selection to avoid the excessive cost of hybridization (Ortiz-Barrientos, Grealy, & Nosil, 2009). This process named reinforcement, eliminates from the populations alleles from individuals with low heterospecific discrimination ability, arising a pattern of reproductive character displacement and strengthening premating isolation in sympatric populations respect to allopatric populations (Ortiz-Barrientos *et al.*, 2009). Additionally, can also produce strengthening of premating isolation between sympatric and allopatric populations of the reinforced species “cascade reinforcement” (Ortiz-Barrientos *et al.*, 2009) (Figure 1D). However, when hybrids are more viable than the parental species, one or both parental species can be locally extinct or can merge (speciation reversal) if reproductive isolation between them is incomplete (Seehausen *et al.*, 2008) (Figure 1E). When hybrids are reproductively isolated from the parental species, they can become a new hybrid lineage [(homoploid, without duplications of the genetic material) or allopolyploid with the duplication of the genetic material) hybridization] (Todesco *et al.*, 2016) (Figure 1E).

2) Literature review on hybridization vulnerability based on Genetic Divergence

2.3) Review of genetic divergence preventing hybridization (insect orders).

2.2) Behavioral versus mechanical barriers

3) Literature review on climate induced range shifts in insects in Europe from last 20 years and most common scenarios increasing [introgression (transfer of adaptations), reinforcement of reproductive isolation, and the origin of new hybrid lineages] or decreasing biodiversity (breakdown of reproductive barriers, species fusion and extinction) during climate-induced range shifts of insects. We will discuss which insects are more prone to hybridize and which are the most common outcomes in each order.

**3.1)** **Hybridization prevalence and vulnerability Lepidopterans**

**3.2) Hybridization prevalence and vulnerability Hymenopterans**

**3.3) Hybridization prevalence and vulnerability Orthopterans**

**4.4) Hybridization prevalence and vulnerability Dipterans**

**4.5 ) Hybridization prevalence and vulnerability in odonates**

Hassall (2015) proposed odonates as candidate macroecological barometers for global climate change. But, why odonates respond drastically to global warming? On the one hand, they respond drastically because of their origin in a geological period characterized by a tropical climate (Carboniferous) and their sensitivity to water conditions (they spend their larval life in aquatic habitats) and on the other hand, they respond drastically because of their high dispersal abilities and generalized niches (Tang & Visconti, 2021). Menendez (Menéndez, 2007) highlights that species responses to climate change depend on the flexibility of different life-history characteristics. In fact, lentic odonate species (those adapted to standing waters) experienced on average a northward range shifts of 115 km per decade, while lentic odonate species (adapted to running waters) did not change their range boundaries (Grewe *et al.*, 2013).

There is plenty of empirical evidence of the high prevalence of interspecific sexual interactions in both, damselflies (Sánchez-Guillén *et al.*, 2014) and dragonflies (see Supporting Information Table S1). Table S1 includes 256 sexual interactions clustered in seven categories: 1) sexual interaction, when species interact without physical contact; 2) tandem attempt, when he male attempts to grasp the female); 3) tandem formation, when tandem is formed; 4) mating, when male and female primary genitalia come into contact); oviposition, when the female involved in the heterospecific copulations lay eggs); 6) hybrids formation in nature, and 7) hybrids formation in the laboratory. Table S1 shows that interspecific sexual interactions happen frequently, and not only between species from the same genus (143 pairs of species involved in intragenic interactions), but also between species from different genera (49 pairs of species involved in intergenic interactions) and from different families (42 pairs of species involved in interfamily interactions). However, although intergenic and interfamily matings are common in both, damselflies and dragonflies, hybrids are only produced when interspecific matings take place between species of the same genus (intragenic) (Supporting Information Table S1 collets 45 hybridization cases between pairs of closely related species of both, damselflies (n=22) and dragonflies (n=23). In fact, when comparing genetic threshold of hybrids occurrence in damselflies (<1.8%) [18] with genetic threshold of hybrids occurrence in other insects such as Drosophila (5%) (Coyne, 2004) or Heliconious butterflies (2-6%) (Mallet, 2005), odonate hybrids are produced between more closely related species, i.e., odonates complete reproductive isolation earlier than other insects, probably because sexual selection is an important force involved in odonate speciation (Wellenreuther & Sánchez-Guillén, 2015). However, although hybrids are not formed, one outcome could be the demographic decline of one species (Sánchez-Guillén *et al.*, 2016).

Table 1 collects empirical evidence for different scenarios increasing [(unidirectional and bidirectional introgression, and reinforcement of reproductive isolation, including evidence of strengthening of reproductive isolation and of reproductive character displacement)] and decreasing (local extinction) genetic divergence and biodiversity during climate-induced range shifts in odonates. I found eight confirmed cases of hybridization, (mostly of unidirectional introgression, but also including reinforcement and local extinction of one parental species), involving European and Japanese odonate species experiencing climate-induced range shifts that can alter the equilibrium of the hybrid regions (sympatric or partially sympatric species). I included also six suspected cases of hybridization detected by Geiger et al. (Geiger *et al.*, 2021), of species experiencing climate-induced range shifts (Table 1). Geiger et al. (Geiger *et al.*, 2021) published a DNA barcode dataset including 103 Central European odonates of which, twelve species (six pairs of species) showed unambiguous identifications due to weak mitochondrial differentiation and partial haplotype sharing, that mostly was explained, by the authors, by recent divergence or possible introgressive hybridization. The increase of the overlapping ranges could alter the stablished equilibrium in the hybrid zone, putting these species are in a vulnerable situation. Additionally, I included another pair of species (in this case allopatric species) vulnerable to hybridization in case that species overlap their distribution.

During range expansions founder effect and genetic drift decrease genetic diversity, but when the expanding species come into contact with a parapatric or allopatric sister species, the loss of genetic diversity can be counteracted by hybridization and introgression (Pfennig, Kelly, & Pierce, 2016). The damselfly *Ischnura elegans*, which has drastically expanded its distribution in Spain in the last 20 years, is an example in damselflies, on how genetic diversity is counteracted by introgression with its sister species, *Ischnura graellsii*. In fact, both, genetic divergence and genetic differentiation of *I. elegans* populations from the north-western Spanish hybrid region were higher than in allopatric populations of *I. elegans* from France (Sánchez-Guillén *et al.*, 2011a; Wellenreuther *et al.*, 2018). However, the advantage of the expanding species is a disadvantage for the autochthonous species. *Ischnura graellsii,* which is a endemism from the Iberian Peninsula*,* is experimenting local extinction in most of the localities occupied by *I. elegans* (Sánchez-Guillén *et al.*, 2011a; Wellenreuther *et al.*, 2018). Another well studied example of hybridization and introgression is between *Cordulegaster boltonii* and *C. trinacriae.* Thesetwo sister dragonflies are increasing their overlapping range in central Italy by the northern shift of *C. trinacriae*. In the sympatric region they hybridize with the unidirectional introgression of genes from the autochthonous *C. boltonii* into the expanding *C. trinacriae* (Solano *et al.*, 2018).

The reinforcement of the reproductive isolation, based on its high prevalence despite the paucity of empirical data, seems to be also common in odonates (see Table 1). Between allopatric *I. elegans* and *I. graellsii,* hybridization is unidirectional, with the introgression of genes from *I. graellsii* into *I. elegans.* Over time in sympatry, this pattern changes, and the introgression becomes bidirectional (Sánchez-Guillén *et al.*; Sánchez-Guillén, Wellenreuther, & Cordero-Rivera, 2011b) emerging a pattern of reinforcement of the reproductive isolation, detected by the strengthening of the reproductive isolation between *I. elegans* and *I. graellsii* (Arce-Valdés, Ballén-Guapacha, & Sánchez-Guillén), and by reproductive character displacement in reproductive traits between *I. elegans* and *I. graellsii* (personal observation, RAS-G)in sympatry. In European Calopteryx *Spp.*, despite the extended sympatry and minor interspecific niche differences, hybridization rates are very low (see Table 1), probably due to large differences in secondary sexual wing traits (Sánchez-Guillén *et al.*, 2014). However, there is empirical evidence of the potential for character displacement in Calopteryx and Heaterina damselflies from north America (Waage, 1975; Anderson & Grether, 2010).

**Research directions** **and conclusions**

Although hybridization outcomes on genetic divergence and biodiversity during climate-induced range shifts are of major interest in conservation biology, and odonates respond drastically to global warming shifting ranges to north, their low vulnerability to hybridize, and the mainly positive outcomes of the hybridization (introgression and reinforcement), make of the European and Japanese odonates a group of little concern. However, in the case of threatened species, there is still a concern by a possibility of the local extinction.

**Tables and Figures**

**Table 1**. Summarize study methods on **climate-induced hybridization** studies in insects. Column 1 indicated whether hybridization is confirmed, suspected, or predicted. Columns 2 and 3 indicate species involved in hybridization.Column 3 indicates range trend (climate-induced range expansions) of involved species. Column 4 indicates hybridization outcome. Column 6 indicates DNA markers applied for species and hybrid identification: nDNA (nuclear genes) and mtDNA (mitochondrial genes); RAPDs (Random Amplified Polymorphic DNA); AFLPs (Amplified-Fragment-Length Polymorphic DNA); SSRs (Simple Sequence Repeats); and SNPs (Single Nucleotide Polymorphisms). Column 7 indicates the level of genetic divergence between involved species.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Hybridization** | **Interacting species** | | **Distribution** | **Range-trend** | **Outcome** | **Genetic marker** | **Genetic divergence** | **Reference** |
| Confirmed hybridizing species | Sp. 1: *Anax imperator* | Sp. 2: *A. partenophe* | Sympatric | Sp1.: moderate  expansion  Sp. 2: moderate  expansion | Hybridization | mtDNA (COI barcoding) | COI: 0.35 | [27] |
| Sp. 1: *Calopteryx splendens* | Sp. 2: *C. virgo* | Sympatric | Sp. 1: moderate  expansion  Sp. 2: moderate  expansion | Unidirectional introgression | SSRs | COI: 9.33 | [27,37] |
| Sp. 1: *Calopteryx splendens* | Sp. 2: *C. haemorroidalis* | Sympatric | Sp. 1: moderate  expansion  Sp. 2: stable | Bidirectional introgression | SSRs | na | [38] |
| Sp. 1: *Cordulegaster trinacriae* | Sp. 2: *C. boltonii* | Largely allopatric, small area of co-occurrence | Sp. 1: expansion  Sp. 2: stable | Unidirectional introgression,  **Reinforcement** | mtDNA, nDNA | COI: 5.37 | [27,31] |
| Sp. 1: *Ischnura elegans* | Sp. 2: *I. graellsii* | Largely allopatric, small area of co-occurrence | Sp. 1.: fast expansion  Sp. 2: contraction | Bidirectional introgression,  **Local extinction**,  **Reinforcement** | SSRs, SNPs | COI: 0 | [29,30,39] |
| Sp. 1: *Ischnura elegans* | Sp. 2: *I. genei* | Largely allopatric, small area of co-occurrence | Sp. 1: stable  Sp. 2: stable | Introgression | SSRs | COI: 0 | [18,27] |
| Sp. 1: *Ischnura senegalensis* | Sp. 2: *I. elegans* | Largely allopatric, small area of co-occurrence | Sp. 1: fast expansion  Sp. 2: stable | Hybridization in laboratory | SSRs | na | [40] |
| Sp. 1: *Mnais costalis* | Sp. 2: *M. pruinosa* | Sympatric | Sp. 1: expansion  Sp. 2: expansion | Introgression,  **Reinforcement** | mtDNA, nDNA | na | [41] |
| Suspected hybridizing species | Sp. 1: *Calopteryx splendens* | Sp. 2: *C. xanthostoma* | Largely allopatric, small area of co-occurrence | Sp. 1: moderate  expansion  Sp. 2: na | Hybridization or recent divergence? | mtDNA (COI barcoding) | COI: 0 | [27,42] |
| Sp. 1: *Chalcolestes parvidens* | Sp. 2: *C. virens* | Largely allopatric, small area of co-occurrence | Sp. 1:na  Sp. 2: na | Hybridization or recent divergence? | mtDNA (COI barcoding) | COI: 8.92 | [27] |
| Sp. 1: *Coenagrion puella* | Sp. 2: *C. pulchellum* | Sympatric | Sp. 1: moderate expansion  Sp. 2: stable | Introgression or recent divergence? | mtDNA (COI barcoding) | COI: 0 | [27] |
| Sp. 1: *Coenagrion puella* | Sp. 2: *C. ornatum* | Sympatric | Sp. 1: moderate expansion  Sp. 2: moderate expansion | Introgression or recent divergence? | mtDNA (COI barcoding) | COI: 0 | [27] |
| Sp. 1: *C. pulchellum* | Sp. 2: *C. ornatum* | Sympatric | Sp. 1: stable  Sp. 2: moderate expansion | Introgression or recent divergence? | mtDNA (COI barcoding) | COI: 0 | [27] |
| Sp.1: *Somatochlora meridionalis* | Sp. 2:  *S. metallica* | Largely allopatric, small area of co-occurrence | Sp. 1: moderate expansion  Sp. 2: na | Hybridization? | mtDNA (COI barcoding) | COI: 0.31 | [27] |
| Predicted hybridizing species | Sp.1: *Gomphus schneiderii* | Sp. 2: *Gomphus vulgatissimus* | Allopatric | Sp. 1: na  Sp. 2: stable | Predicted hybridization | mtDNA (COI barcoding) | COI: 0.72 | [27] |

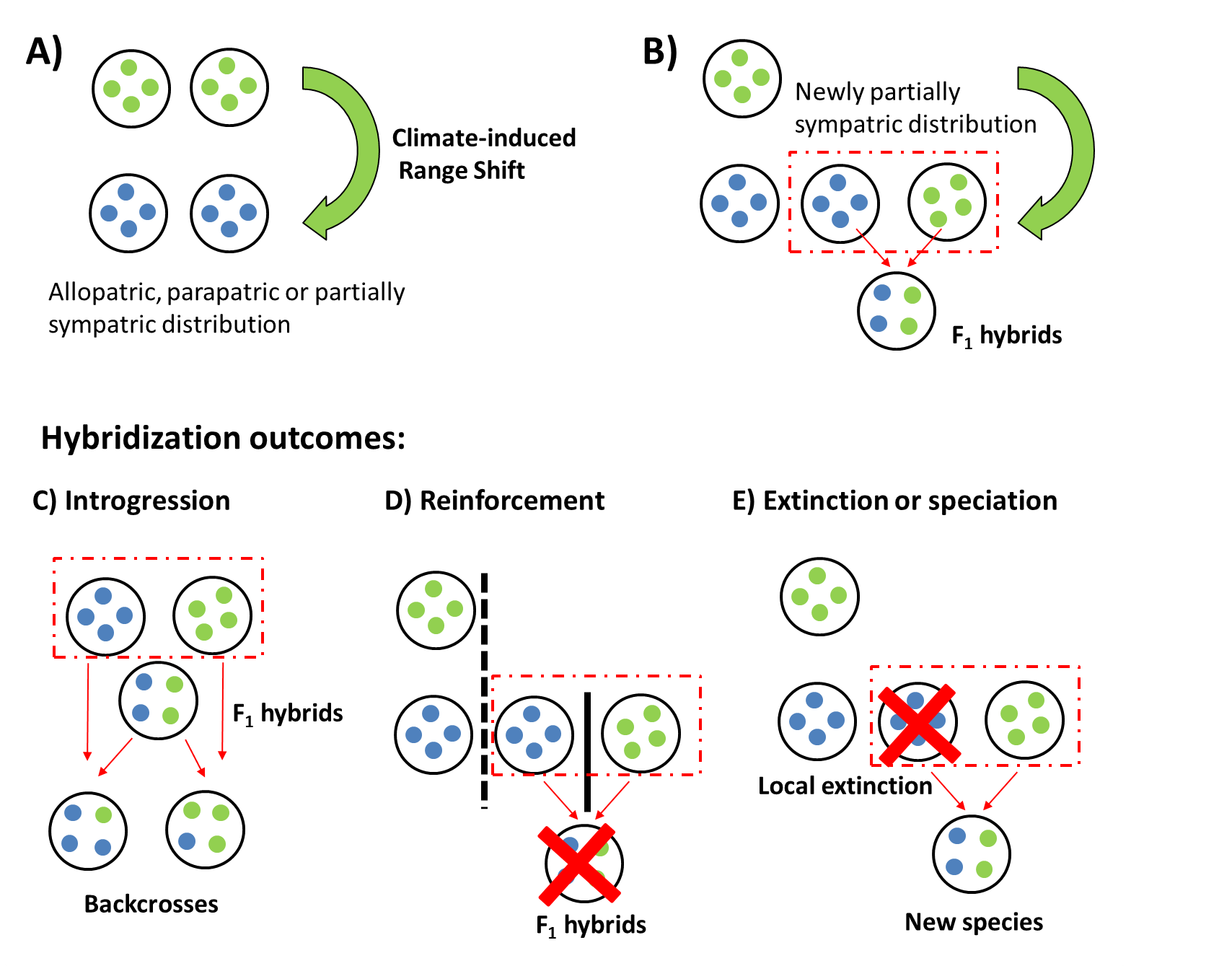
Note: These studies were found in a systematic literature search of Google Scholar and the Web of Science, using the combined search terms:

‘climate-change AND odonates\*’OR ‘climate-change AND damselflies\*’ OR ‘climate-change AND dragonflies\*’ OR ‘hybridization AND odonates\*’ OR ‘hybridization AND odonates\*

**Table 2.** Studies showing different introgressive hybridization outcomes following range shifts in Lepidopterans, Hymenopterans, Orthopterans and Dipterans.

**Figure 1.** Genetic threshold of hybrids occurrence:correlation between genetic divergence and reproductive isolation.

**Figure 2. Common outcomes following climate-induced hybridization.** **A**) Allopatric species come into secondary contact due to climate-induced norther range shifts. **B**) Species, that have evolved allopatry and have incomplete reproductive isolation between, will produce hybrids. **C**) Different hybridization patterns develop depending on intrinsic and extrinsic factors; most common scenarios in odonates are adaptive introgression, reinforcement and extinction or speciation. Circles represent populations of individuals; blue or green circles are parental populations, while blue-green circles represent hybrid individuals of mixed ancestry. Small red arrows denote crosses, and red cross indicate selection against (hybrid or parental individuals).



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