A standardised framework for classifying estimates of reproductive isolation across the Tree of Life

Jonah Walker[1,2]*, Chris Cooney[3], Jonna Kulmuni[4,5], Konrad Lohse[6], Joana I. Meier[1,2], Richard M. Merrill[7], Elizabeth Scordato[8], Carole M. Smadja[9], Sonal Singhal[10]+, Roger Butlin[3,11]+, Sean Stankowski[12]*+

1. Department of Zoology, University of Cambridge, CB2 3EJ, Cambridge, UK
2. Tree of Life Programme, Wellcome Sanger Institute, Hinxton, CB10 1RQ, Cambridgeshire, UK
3. Ecology and Evolutionary Biology, School of Bioscience, University of Sheffield, Sheffield, S10 2TN, United Kingdom
4. Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Science Park 904, Amsterdam, Netherlands
5. Organismal and Evolutionary BIology, University of Helsinki, Viikinkaari 1, 00790 Helsinki, Finland
6. Institute of Ecology and Evolution, University of Edinburgh, Ashworth Laboratories, Charlotte Auerbach Road, Edinburgh EH9 3FL, UK
7. Division of Evolutionary Biology, LMU Munich, Grosshaderner Straße 2, 82152, Planegg-Martinsried, Germany
8. Department of Biological Sciences, California State Polytechnic University, Pomona, CA, 91768
9. Institut des Sciences de l'Evolution de Montpellier (ISEM), Univ Montpellier, CNRS, IRD, Montpellier, France
10. Department of Biology, California State University - Dominguez Hills, Carson, CA, United States, 90747
11. Department of Marine Sciences, University of Gothenburg, Tjärnö Marine Laboratory, 45296 Strömstad, Sweden
12. Department of Ecology and Evolution, The University of Sussex, Brighton, UK

* corresponding authors; Jonah Walker (jmw258@cam.ac.uk) & Sean Stankowski (Sean Stankowski (S.Stankowski@sussex.ac.uk)
+ equal contributions

Abstract

Understanding reproductive isolation (RI) between lineages is a central goal of speciation research. While the strength of RI has been estimated across a broad range of taxa, synthesising these data remains challenging, partially because we lack a common language for classifying and reporting RI estimates. Here, we present the *Reproductive Isolation Ontology* (RIO), a structured framework designed to standardise the classification of RI estimates across the Tree of Life. The RIO comprises three interrelated domains, each organised into a nested hierarchy of classification terms. We demonstrate the utility of this approach and call for researchers to adopt the RIO to facilitate the integration of speciation research.

Why we need a standard language for reproductive isolation

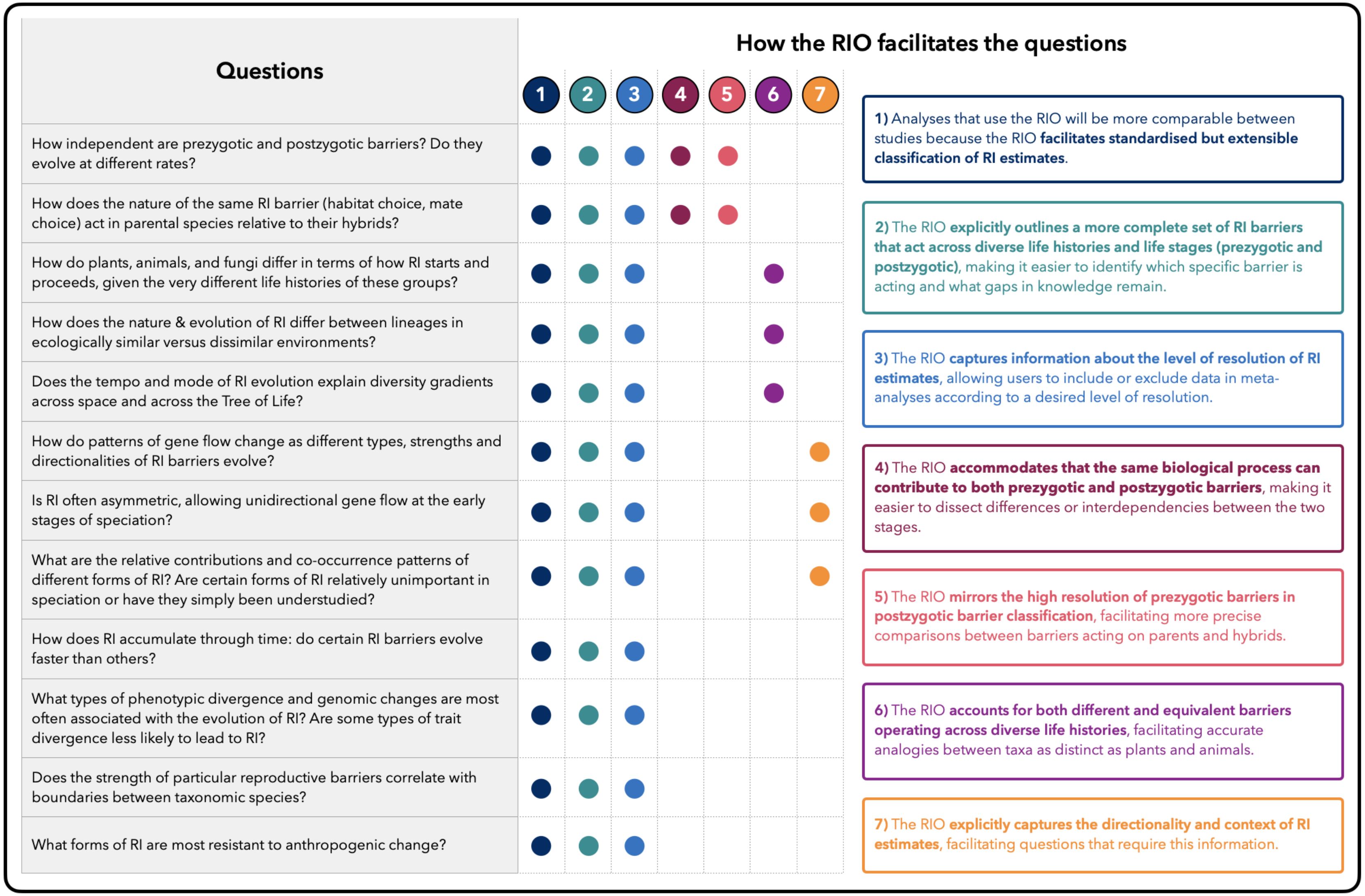
When two lineages meet – whether naturally or via human manipulation – their futures will be shaped by reproductive barriers between them. These barriers come in a diversity of forms, including differences in habitat preference and reduced fitness of hybrids, all of which reduce the production of fertile offspring between lineages. The total of these barriers comprises reproductive isolation (RI) (Westram *et al.*, 2022). As the strength of RI between lineages increases, gene flow is expected to decrease, thus further promoting lineage divergence. Because RI both reflects the extent of divergence between lineages and the likelihood of further divergence, quantifying RI has become central to studying speciation (Dobzhansky, 1937a).

Most studies have explored the role of RI in speciation by pursuing focused analyses in a single clade. These studies often result in numerical estimates of RI strength between specific pairs of lineages, along with metadata on the lineages (e.g., degree of ecological or genetic divergence) and a classification of RI components (e.g., habitat preference, hybrid sterility). Comparative work has synthesized estimates of RI from these studies, attempting to uncover more general patterns about the tempo and mode of RI evolution and its role in species diversification. For example, studies have shown that intrinsic postzygotic barriers can be particularly effective in limiting gene flow early in speciation (Coughlan & Matute, 2020), that ecological divergence predicts the extent of reproductive isolation (Funk *et al.*, 2006), and that the rate of RI evolution does not predict macroevolutionary speciation rate variation in fruit flies or birds (Rabosky & Matute, 2013).

Comparative studies are still few in number and limited in scope, partially because comparative datasets on RI are scarce. Thus, it is unclear whether these findings will hold across more diverse sets of taxa, and many further questions about the role of RI in speciation remain unanswered. Filling these gaps requires datasets that compile standardised estimates of RI across a large and diverse set of taxa, but empirical estimates of RI are collected using multiple methods and approaches, at different levels of resolution, with terminology that varies across study systems. Synthesising across this heterogeneity is non-trivial because it requires 1) a standardised approach to estimating the strength of RI components that is comparable across different study designs and taxonomic groups; and 2) a standardised language for classifying these RI components to ensure equivalent barriers are compared. Sobel and Chen (2014) proposed a set of equations that allow RI component strength to be calculated and compared across diverse taxa (i.e., the RI4 index); these measures of RI have since been widely adopted. However, while various authors have developed their own schema to classify RI components across studies (e.g. (Lowry *et al.*, 2008; Hernández-Hernández *et al.*, 2021; Christie *et al.*, 2022; Garlovsky *et al.*, 2024)), no standardised or consensus approach to classifying RI components has been widely adopted, either for individual systems or meta-analyses.

In 2021, we began collating RI data from case studies in many different clades to understand how RI evolves across the Tree of Life. Through this process, we identified four primary challenges in classifying individual estimates of RI. RI classifications must accommodate (1) the diversity of organismal life histories, (2) the differing resolutions at which RI estimates are made, (3) the nuances of postzygotic isolation, and (4) the multiple dimensions of RI. Building on the schema outlined by previous researchers and endeavouring to address these challenges, we propose a standardised framework to classify and categorise RI: the Reproductive Isolation Ontology (RIO). Here, we discuss how the RIO resolves these challenges, thus enabling us to create comparable datasets across diverse taxa to better understand the role of RI In speciation (Figure 1).

Figure 1: Questions about the role of reproductive isolation (RI) in speciation that the Reproductive Isolation Ontology (RIO) can help address.



How the RIO facilitates the questions

Questions	1	2	3	4	5	6	7
How independent are prezygotic and postzygotic barriers? Do they evolve at different rates?	●	●	●	●	●		
How does the nature of the same RI barrier (habitat choice, mate choice) act in parental species relative to their hybrids?	●	●	●	●	●		
How do plants, animals, and fungi differ in terms of how RI starts and proceeds, given the very different life histories of these groups?	●	●	●			●	
How does the nature & evolution of RI differ between lineages in ecologically similar versus dissimilar environments?	●	●	●			●	
Does the tempo and mode of RI evolution explain diversity gradients across space and across the Tree of Life?	●	●	●			●	
How do patterns of gene flow change as different types, strengths and directionalities of RI barriers evolve?	●	●	●				●
Is RI often asymmetric, allowing unidirectional gene flow at the early stages of speciation?	●	●	●				●
What are the relative contributions and co-occurrence patterns of different forms of RI? Are certain forms of RI relatively unimportant in speciation or have they simply been understudied?	●	●	●				●
How does RI accumulate through time: do certain RI barriers evolve faster than others?	●	●	●				
What types of phenotypic divergence and genomic changes are most often associated with the evolution of RI? Are some types of trait divergence less likely to lead to RI?	●	●	●				
Does the strength of particular reproductive barriers correlate with boundaries between taxonomic species?	●	●	●				
What forms of RI are most resistant to anthropogenic change?	●	●	●				

1) Analyses that use the RIO will be more comparable between studies because the RIO **facilitates standardised but extensible classification of RI estimates**.

2) The RIO **explicitly outlines a more complete set of RI barriers that act across diverse life histories and life stages (prezygotic and postzygotic)**, making it easier to identify which specific barrier is acting and what gaps in knowledge remain.

3) The RIO **captures information about the level of resolution of RI estimates**, allowing users to include or exclude data in meta-analyses according to a desired level of resolution.

4) The RIO **accommodates that the same biological process can contribute to both prezygotic and postzygotic barriers**, making it easier to dissect differences or interdependencies between the two stages.

5) The RIO **mirrors the high resolution of prezygotic barriers in postzygotic barrier classification**, facilitating more precise comparisons between barriers acting on parents and hybrids.

6) The RIO **accounts for both different and equivalent barriers operating across diverse life histories**, facilitating accurate analogies between taxa as distinct as plants and animals.

7) The RIO **explicitly captures the directionality and context of RI estimates**, facilitating questions that require this information.

Challenges of classifying RI estimates within existing schemes

Most modern classifications of RI are modifications of the structure originally proposed by Dobzhansky (1937b). This envisions RI as a sequence of successive barriers, each of which progressively reduces the likelihood of successful reproduction (Figure 2A). For example, consider two plant lineages with low levels of cross-pollination because they occur in largely non-overlapping geographic ranges. When cross-pollination does occur, hybrids rarely form because gametes are incompatible. The few hybrids that do form have low survival rates because their physiology is mismatched to their environment. The strengths of these three barriers could be estimated empirically and would be classified as “geographic isolation”, “fertilisation isolation”, and “hybrid inviability”, respectively; Figure 2A. While this basic structure has facilitated decades of fruitful RI research, we have identified four complications that make it challenging to classify and compare RI estimates with this approach.

Challenge 1: Existing classifications of RI exclude some organisms

Most existing classifications are designed around a typical animal or plant life cycle, in which individuals are born, disperse, exchange gametes, and reproduce. Many organisms do not follow this pattern, and even for animals and plants, classifying reproductive barriers in a comparable and biologically meaningful manner can be difficult (Figure 2B). For instance, plants and broadcast-spawning animals (e.g. corals) do not ‘mate’ but disperse their gametes, so terms like *pre-mating* and *post-mating* are somewhat inappropriate. We could create separate frameworks for plants, broadcast-spawning animals, and internally fertilising animals, but we would then be unable to analogize across different terms, limiting comparative analyses across these taxa. In addition, some classifications include highly taxon-specific terms like "sperm competition" or "pollen-pistil interactions" that lack clearly defined equivalents in other taxonomic groups. To address these limitations, we need a classification that (i) accommodates a wide range of life histories, (ii) avoids lumping non-equivalent barriers together, and (iii) meaningfully groups equivalent processes across taxa.

Challenge 2: Varying resolution complicates classifying barriers within a single term

In existing schemes, each RI estimate is classified as a single component within a clear chronological sequence (Figure 2A). However, estimates of RI strength cannot always be easily ascribed to a single component (Figure 2C). As an example, take the case of two plant lineages that rarely produce hybrid seeds. The low yield of hybrid seed could be because of pollen rejection (a pre-zygotic barrier that acts after the transfer of gametes) and/or low viability of hybrid embryos (a post-zygotic barrier). A study that only measures the relative yield of hybrid seed cannot distinguish between these two barrier components of RI and would need an RI classification that reflects this uncertainty. In contrast, a study that estimates the relative roles of pollen rejection and seed viability in reducing hybrid seed yield could apply a more precise RI classification (Garlovsky *et al.*, 2024).

As another example, consider two bird species adapted to different altitudes. Suppose that a high-altitude individual ends up at low altitude and hybridises with a low-altitude species. The resulting hybrids have low survival, which could simply be recorded as 'hybrid inviability'. But imagine further that this low survivorship is known to be partially due to reduced parental provisioning by the high-altitude parent struggling with the heat of the low-altitude habitat. The ecological maladaptation of parents exists before the formation of hybrid offspring and is a form of 'immigrant inviability', but reduced provisioning rate only manifests after fertilisation through the poor performance of a hybrid. Its complexity, and the fact that it results from both prezygotic and postzygotic factors, means that this barrier estimate cannot be neatly ascribed to a single RI term. Further experimental work would be required to separate the different contributions of ‘immigrant inviability’ and ‘hybrid inviability’, thus increasing the resolution of the RI estimate.

These two examples exemplify how the complexity of RI barriers can result in variation in the resolution at which they are recorded. Moreover, even if researchers measure RI at higher resolution, they may also choose to merge estimates of different barriers in order to estimate ‘total isolation’, ‘prezygotic isolation’, or ‘postzygotic isolation’. Comparing RI components across diverse studies thus requires a classification system that accounts for different levels of resolution to compare like with like. Developing a method of RI classification that explicitly accommodates different levels of resolution would enable a more functional description of empirical estimates of RI.

Challenge 3: Diverse postzygotic barriers are often ignored or lumped under imprecise terms

Prezygotic barriers are typically finely subdivided. For example, researchers regularly distinguish between habitat, temporal, pollinator, behavioural, and mechanical components of prezygotic RI (Dobzhansky, 1937b; Mayr, 1963; Coyne & Orr, 2004). By contrast, postzygotic barriers are typically lumped into a few broad categories — i.e., ‘hybrid sterility’ or ‘hybrid inviability’ — even though postzygotic barriers mirror prezygotic barriers in their number and diversity (Figure 2D). For example, a hybrid might be effectively sterile for multiple reasons, including phenological divergence that reduces overlap in breeding season (a form of temporal isolation) or morphological divergence in reproductive organs that impairs successful copulation (a form of mechanical isolation). Further, under some existing schema, these barriers would be classified as prezygotic – even though they act on the hybrid – and would be lumped together with barriers acting on parental species.

This lack of precision has hampered our ability to distinguish among fundamentally different forms of postzygotic isolation. As a result, certain components of RI are under-represented or remain largely unexplored, preventing us from addressing fundamental questions about the evolution and nature of postzygotic isolation ((Matute & Cooper, 2021); Fig. 1). To overcome these limitations, a revised classification of reproductive isolation would ideally expand the terminology used for postzygotic barriers to mirror the precision typically used for prezygotic barriers, with comparable levels of resolution. This classification will allow us to understand how parental barriers can be broken or reshaped in hybrids, thus influencing hybrids' ability to act as a bridge to gene flow between parental species.

Challenge 4: RI estimates have multiple dimensions

Empirical estimates of RI often focus on the specific reproductive barrier they are measuring and its strength. However, RI estimates have other important dimensions that provide context about these barriers and are essential for their accurate interpretation (Figure 2E). For example, RI can be asymmetric, such that one lineage will more readily reproduce with another than vice versa. Asymmetry in RI is expected under several scenarios, including the evolution of unidirectional genetic incompatibilities (Turelli & Moyle, 2007) or as a result of reinforcement selection (Yukilevich, 2012). Thus, capturing the directionality of RI is important. Additionally, RI is measured in different environments, such as in the laboratory, under common garden greenhouse conditions or in the wild. The strength of the same barrier might differ across environments (Rundle, 2002), so RI strength should be recorded relative to the environment in which it is measured. Thus, a revised classification of reproductive isolation should capture the multiple dimensions of RI.

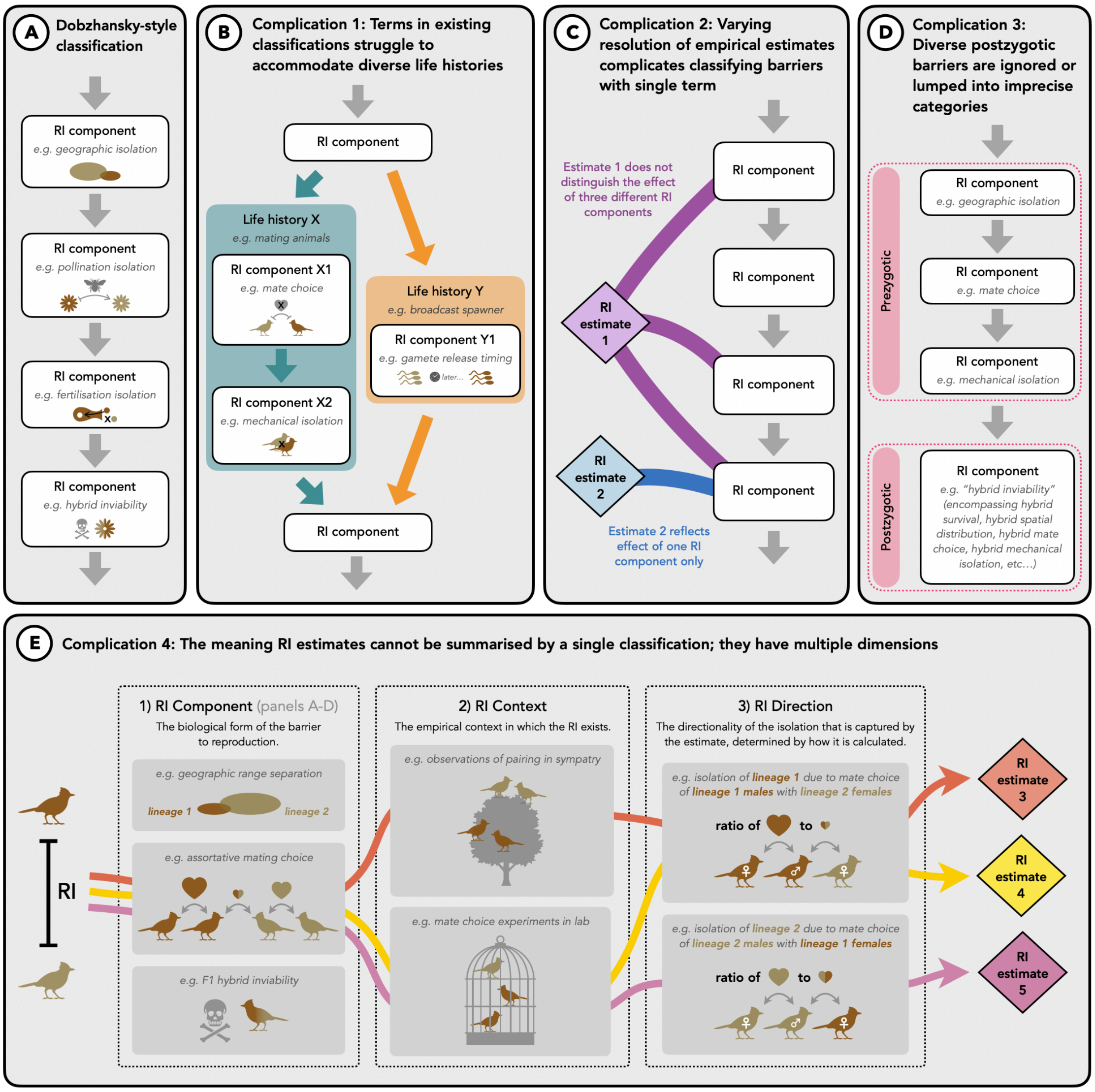


Figure 2: The current basic structure for classifying reproductive isolation (RI) and its complications. (**A**) Most existing schema envisage RI as a sequence of successive barriers, each of which progressively acts to reduce the likelihood of reproduction. RI estimates would be classified by selecting the most appropriate barrier in this sequence (e.g. 'geographic isolation'). This schema faces several complications: (**B**) existing barrier classifications cannot be easily applied across a diversity of life histories; (**C**) varying resolution of estimates makes it challenging to classify them with a single term; (**D**) diverse postzygotic barriers are typically overlooked or 'lumped' into imprecise categories; and (**E**) RI has multiple dimensions beyond the barrier component and its strength.

Our proposal: an ontological classification for RI estimates

Our goal is to provide a precise but flexible framework for classifying estimates of RI that addresses the challenges outlined above. Taking inspiration from the Gene Ontology used in molecular biology, we propose the Reproductive Isolation Ontology (‘RIO’; Figure 3). The RIO comprises controlled vocabularies in three ‘domains’ — RI Component, RI Direction, and RI Context — each of which captures a different facet of empirical RI estimates, as discussed below (see Supplementary Information for a full description of all RIO terms). Importantly, while we have defined these three domains, which we believe capture the most salient features of an RI estimate, the RIO can be expanded to include other domains, or to add terms in any domain. In practice, any estimate of RI strength can be classified by selecting one term from each of the domains (see Table S1 for several worked examples).

RI Component classifies an RI estimate according to the biological basis of the isolating barrier. This domain has been designed to offer detailed standardized language for RI components, to allow differing levels of study resolution, to accommodate a diversity of life histories, and to fully capture the nuances of postzygotic isolation.

Existing RI schema already group different RI barriers into hierarchies – for example, temporal, behavioral, and habitat isolation are all nested within prezygotic isolation, and hybrid sterility and hybrid inviability are specific types of postzygotic isolation (Coyne & Orr, 2004). Here, we build on this foundation to create a more detailed nested hierarchy (Figure 3A). For example, most existing classifications categorise temporal isolation as a form of prezygotic isolation, as we do here. The RIO further dissects prezygotic isolation into types that act before lineages interact (“pre-encounter”) – among which temporal isolation is included – and those that act after lineages interact (“post-encounter”). Additionally, we categorize temporal isolation more finely – i.e., species can be isolated because they keep different “seasonal” or “diurnal” schedules. This more detailed hierarchy better captures the diversity of RI barriers seen in the wild.

Further, because this hierarchy is nested, it can accommodate differing levels of resolution across different RI estimates (cf. Challenge 2). Imagine a system in which species rarely mate because they are rarely found in the same habitats. The two species might occur in different habitats because they have different physiological requirements (“habitat suitability”) and/or one species actively disperses into a distinct habitat from the other (“habitat preference”). If a study cannot distinguish between the two sources of spatial segregation, they can describe the pattern coarsely using the parent term of “habitat preference” and “habitat suitability”: “spatial”. This nested hierarchical structure – combined with the unique alphanumeric codes for each term (Table S1) – will also facilitate meta-analyses; researchers will be able to either select only those studies conducted at the relevant level of resolution or collapse studies down to more coarse categories.

Additionally, we have worded RI Component terms carefully to accommodate a diversity of reproductive life histories (cf. Challenge 1). For example, the life-stage between meiosis and fertilization is typically referred to as ‘gametes’ in animals, whereas for sporophyte-dominant plants, this life-stage encompasses spores, gametophytes (pollen and ovule), and gametes. We use the taxon-agnostic term ‘meiotic products’ to encompass this diversity of terms. This more generalized terminology will facilitate comparisons of RI Components acting on the same life stage across diverse life histories. At the same time, we recognize that RI components function differently in different life histories. Some plants rely on wind or insects for cross-pollination, some broadcast-spawning animals similarly rely on ocean currents for cross-fertilization, and many animals require mating for cross-fertilization. Because these systems are decidedly distinct and are subject to different selective pressures, we distinguish them as *“Vector transfer of meiotic products”* (e.g. between insect/wind-pollinated plants or broadcast-spawning animals) and *“Pairing transfer of meiotic products”* (e.g. between animals that mate). More broadly, our choice of language ensures that diverse estimates of RI are classified accurately and can be compiled in syntheses.

Finally, RIO expands the postzygotic terms in the RI Component classification to mirror the prezygotic terms, enabling postzygotic estimates of RI to be classified to the same precision as prezygotic estimates (cf. Challenge 3). For example, estimates of mate choice between two animal lineages would be categorized as the prezygotic term *“Pairing association”*. If we then measure mate choice of F1 hybrids relative to their parents or to each other, we can categorize those estimates as the postzygotic term *“F1 pairing association”*. This finer dissection of terms is particularly important for taxa where hybrids are viable and can act as vehicles of gene flow between parental taxa, and we hope it will stimulate research on more diverse and often overlooked forms of postzygotic isolation (over and beyond “inviability” and “sterility”). The RIO is currently designed to accommodate only F1 hybrids; interactions involving later-stage hybrid classes cannot be currently accommodated. However, the flexibility of the RIO means (1) if a hybrid lineage is stable, then it can be represented as a parental lineage in the RIO and its interactions can be catalogued and (2) future researchers can expand the RIO to be more inclusive of other hybrid classes.

RI Direction (Figure 3B) refines an RI estimate according to its directionality. Many aspects of RI are expected to be asymmetric (Orr, 1997; Turelli & Moyle, 2007; Yukilevich, 2012); for example, Haldane’s Rule predicts that the heterogametic sex will evolve hybrid inviability and sterility before the homogametic sex. By capturing the directionality of RI, we will be better able to understand how asymmetry in RI evolves and impacts lineage divergence. *RI Context* (Figure 3C) both categorizes how RI was measured – i.e., whether by observation, experiment in a controlled setting, or experiment in a natural habitat – and how the environment relates to the natural environments of the lineages under study. The strength and direction of RI may vary depending on the environment where it is measured (Hatfield & Schluter, 1999; Rundle, 2002), and understanding when RI is dependent on the environment might help us predict the stability of species barriers in changing environments. RI Direction and RI Context together allow a more holistic classification of RI, providing greater context to our understanding of RI composition, strength and evolution (cf. Challenge 4). Like the terms for RI Components, these domains are organised in a nested hierarchy using terms that should work for a diversity of taxa.

Applications, adoption, and outlook of the RIO framework

The Reproductive Isolation Ontology (Figure 3) provides a standardised and extensible language for categorising RI. Combined with advances in standardising the calculation of RI strength (Sobel & Chen, 2014), the RIO will help us bring together RI data from case studies across the Tree of Life to better understand speciation (Fig. 1). Further integration of speciation research will require additional work to standardise the reporting of metadata associated with RI estimates, such as the nature and degree of ecological and genetic divergence between the lineages being considered. However, we believe that the RIO represents a concrete step towards facilitating the comparative analyses required to integrate speciation research (see Box 1 for a case study of its utility).

The long-term utility of the RIO will depend both on it being applied retrospectively to the wealth of existing RI datasets, as well as its adoption in future studies. Other biological ontology initiatives (such as the Gene Ontology) have succeeded when the community has recommended modifications where required and adopted using the ontology as a normal part of research culture (Bard & Rhee, 2004). We therefore encourage speciation researchers to both engage with the RIO and report their RI data with associated RIO terms. We invite the research community to learn more about the RIO, to provide feedback, and to suggest modifications by visiting our website: https://www.speciation-rio.info/. This website will maintain an updated, version-controlled version of the RIO for future researchers to adopt.

Ultimately, using the RIO will help researchers to adhere to the FAIR Principles of data stewardship, which call for data to be findable, accessible, interoperable, and reusable (Wilkinson et al., 2016). By reporting RI data with RIO terms, researchers will ensure that their findings can be properly interpreted, be included in large meta-analyses, and ultimately contribute towards answering enduring questions about the origins of biodiversity.

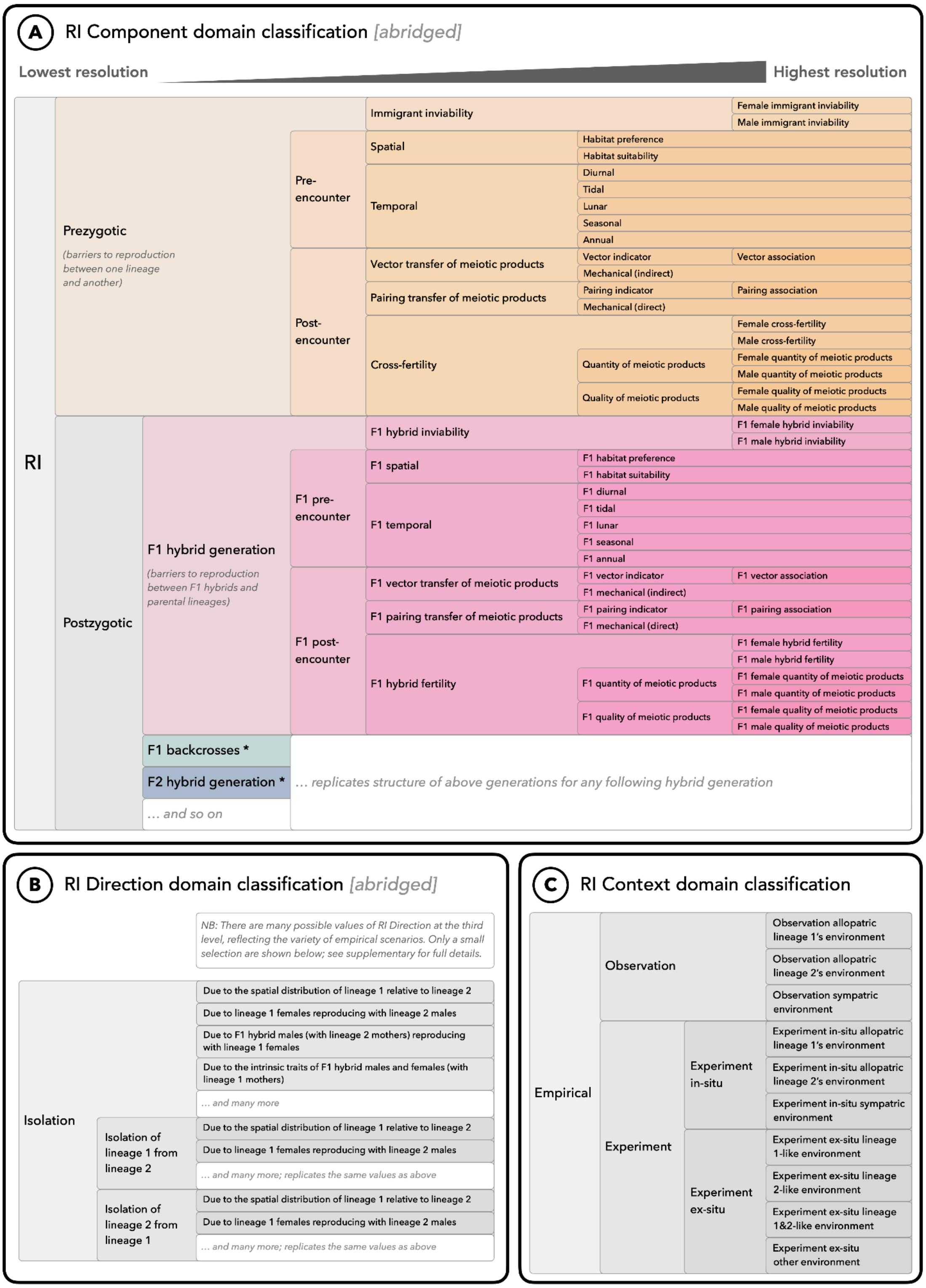


Figure 3: The Reproductive Isolation Ontology (RIO) consists of three classifications or 'domains': (**A**) 'Component', or the type of barrier to reproduction; (**B**) 'Direction', or the directionality of the barrier measurement; and (**C**) 'Context', or the nature of the experimental and observational study used to measure the barrier. The hierarchical structure of the RIO allows researchers to identify the appropriate resolution for their study. Supplemental Information outlines the full terms included in the RIO. *= For simplicity, the

first version of the RIO only accommodates postzygotic RI measurements that involve F1s, but future versions can accommodate a greater range of hybrid generations.

Box 1: Case Study: How would the RIO change a comparative study?

Let's do a thought experiment of how the RIO might facilitate answering a classic question about reproductive isolation (RI): which evolves faster, prezygotic or postzygotic RI (Coyne & Orr, 1989)? The study will be conducted across the diversity of species in which the strengths of different prezygotic and postzygotic barriers have been estimated — including plants, fungi, and animals.

Without the RIO

Following a literature search, we would extract RI barrier type and RI strength from many studies. Unless we implemented a finer resolution scheme for RI like that used in Christie *et al*. (2022), we would classify different barriers coarsely as either prezygotic or postzygotic based on the authors' descriptions. We would additionally survey the literature to associate RI components with metadata like divergence time between lineages and their geographic origins. Ultimately, we would regress prezygotic RI and postzygotic RI strength against divergence time to address our question. If our analyses revealed unexpected results, the coarseness of the dataset would make it challenging to understand the underlying causes. In the future, researchers revisiting this analysis would need to reconstruct our classification scheme to expand or refine the dataset.

With the RIO

Following a literature search, we would extract RI estimates from studies. Some researchers would have associated RIO terms with their estimates, expediting our review; we would be able to classify the remaining studies in a standardised and reproducible way using the RIO. After collecting metadata, the nested hierarchy of the RIO would allow us to collapse all RIO terms to the broadest resolution: 'prezygotic' versus 'postzygotic'. We would then analyse these prezygotic and postzygotic measurements against divergence time to answer the question. If we found unexpected results, we could use the additional data embedded in the RIO to understand why – for example, is the biological basis of prezygotic barriers also reflected in the biological basis of postzygotic barriers? Do asymmetries in prezygotic versus postzygotic isolation affect the rate at which they evolve? Later, researchers will be able to easily extend these analyses by applying the same RIO classification to new datasets.

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