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INVITED COMMENTARY

Using neutral loci to quantify reproductive isolation and speciation: a commentary on Westram et al., 2022

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The formation and persistence of species has been a central focus of biological research (Coyne & Orr, 2004; Dasmahapatra et al., 2010; Dobzhansky, 1937; Seehausen, 2006) because it lies at the heart of how speciation generates biodiversity and how genetic variation is partitioned in nature. The genetic underpinnings and dynamics of speciation have garnered intense debate, and because of this debate, there are several commonly accepted species concepts. The biological species concept, or the idea that a species is a group of interbreeding natural populations that are reproductively isolated from other similar groups (Coyne & Orr, 2004; Dobzhansky, 1937), is arguably the most popular species concept because it connects the existence of discrete genetic clusters with the mechanism that keeps species apart. Reproductive isolation (RI) is central to our understanding of the biology of naturally occurring groups and has been examined in a wide range of taxa, including viruses (Duffy et al., 2007), yeast (Hou et al., 2015), monkeyflowers (Coughlan et al., 2020), *Drosophila* (Cooper et al., 2018; Orr, 2005; Orr et al., 2007), butterflies (McBride & Singer, 2010), stickleback (Thompson et al., 2022) and birds (Delmore & Irwin, 2014).

Identifying the amount of RI sufficient to prevent gene flow, and consequently to identify species boundaries, has been challenging (Roux et al., 2016). This is because speciation is a process during which both RI and gene flow can change continuously, with no clear boundary as to when exchange of genetic information between groups has ceased. As a result, speciation has a critical role in maintaining biodiversity (Rhymer & Simberloff, 1996), but not a limiting one (Harvey et al., 2019; Rabosky, 2016; Rabosky & Matute, 2013). The process of speciation can occur gradually via the accumulation of epistatic incompatibilities between populations (Dobzhansky, 1937) or it can occur nearly instantly, for example via ploidy changes (Otto & Whitton, 2000; Soltis et al., 2015). There has been intense effort trying to determine the primary modes by which speciation occurs (Chesser & Zink, 1994; Fitzpatrick et al., 2009; Jackson & Cheetham, 1999; Templeton, 1981). Similarly, the relative contribution of different types of barriers to gene flow: prezygotic vs. postzygotic (Coyne & Orr, 1989, 1997; Kozak et al., 2012); ecological vs non-ecological speciation (Rundell & Price, 2009; Schluter, 2009) have, and continue to be, intensely debated (Coughlan & Matute, 2020; Fitzpatrick et al., 2008; Mallet et al., 2009; Rundle & Nosil, 2005; Sobel et al., 2010). In part, the difficulties of parsing out reproductive isolation are precisely because it lies at the interface of microevolutionary and macroevolutionary processes. RI requires microevolutionary changes within/between populations that lead to macroevolutionary patterns. The precise timing of this switch between micro- and macroevolutionary changes (i.e. when RI is sufficient to have created a novel species) is debated, difficult to define (Roux et al., 2016) and potentially not biologically significant (Galtier, 2019; Shaw & Mullen, 2014; Stankowski & Ravinet, 2021). All of these factors contribute to the difficulty in studying and identifying speciation in natural systems. Westram et al. (2022) argue that a clear understanding of RI which is universally accepted by the field is a prerequisite to address questions about the generalities of speciation as a process and a driver of diversity. If reproductive isolation is not consistently defined in the field, the synthesis of theoretical and empirical findings across taxa will either be impossible or be misinformative. In their paper, Westram and co-authors set out to (a) summarize the field's understanding of RI, (b) propose a new, inclusive definition of RI for the field and (c) propose methods of calculating RI between taxa.

1 | REPRODUCTIVE ISOLATION LACKS A UNIFIED CONCEPTUAL APPROACH

Collecting genetic data is not a limiting factor in speciation genetics anymore; the main challenge has become the analytical difficulties in discerning evolutionary processes from multidimensional data. The first step for this task is to develop a common language across experimental systems. To date, there has been no systematic assessment of the perception of how scientists perceive and define reproductive isolation. Westram et al. (2022) bridged this gap by surveying 231 speciation researchers to examine the field's understanding and interpretation of RI. They asked these researchers 'In a sentence or two, what is reproductive isolation?' The authors then divided respondent answers into separate categories: (a) organismal (patterns of interbreeding); (b) genetic (patterns of gene flow); (c) permanent distinctiveness of populations; or (d) a combination of the prior three. Broadly speaking, they found that most researchers' views of RI fall into two main camps, which they describe as an organismal (i.e. a reduction in either the production of hybrids, or the reduction in hybrid fitness; 42% of respondents) or genetic (i.e. a reduction in gene flow; 30% of respondents) focus. Some researchers (17%) described RI in a fashion that intermingled Westram and colleagues' 'organismal' and 'genetic' focus, and 6% focussed on the ability of populations to remain distinct. Five per cent of responses were not classified in these categories. Based on the results of this survey, the authors note that researchers are divided on what RI is and how to define it, and as a result, the field needs a clarification to unite our thinking and push the field forward. This realization represents the starting point to provide the foundation for a common language in the field of speciation genetics at the stage in which we have the potential to integrate genomic, genetic and phenotypic data into a synthetic approach.

2 | THE WESTRAM ET AL. SOLUTION

Genomics has the potential to help identify the alleles that are involved in RI phenomena. Under this framework, instead of focussing on measuring the strength of particular barriers, one can measure the extent of gene exchange in the genome (Cruickshank & Hahn, 2014; Noor & Bennett, 2009; Turner et al., 2005; Turner & Hahn, 2010; Wolf & Ellegren, 2017). Loci that have experienced strong divergent selection (Coughlan et al., 2021; Sousa-Neves & Rosas, 2010) and pairs of alleles that are rarely found together in admixed individuals (Powell et al., 2020; Schumer et al., 2014; Schumer & Brandvain, 2016) reduce gene flow between populations. These loci include both intrinsic and extrinsic incompatibilities. In their paper, Westram et al. (2022) propose that RI is a 'quantitative measure of the effect of genetic differences on gene flow [and] compares the flow of neutral alleles from one population to another population, given a set of genetic differences that reduce gene flow, with the flow expected in the absence of any such differences'. This definition encompasses the multiple facets of speciation that spans both the organismal realm (specific traits that reduce gene flow) and the genetic components (the reduction in gene flow derived from causative genetic agents) that underlie RI. This proposal builds on species concepts that leverage genetic information and genetic discontinuities to detect species boundaries, including the phylogenetic species concept (Taylor et al., 2000) and the genetic cohesion concept (Hausdorf, 2011; Templeton, 1989), among many others (reviewed in Coyne & Orr, 2004).

As Westram et al. (2022) define RI as a quantitative measure of the reduction in gene flow in neutral alleles, they also propose distinguishing between local RI at particular loci and genome-wide RI integrating tools already available in the field. By their definition, RI sensu stricto refers to 'genome-wide' RI, that is a single measure for all neutral loci along the genome. Westram et al. argue that measures of RI should generally be genome-wide (instead of at individual loci), and that any comparisons between taxa should be made at this level, barring specific fine-scale questions. To account for instances in which authors describe RI as the effective migration rate (m_e) across the genome, they refer to 'local' RI. Local RI examines loci in which neutral alleles are closely linked to regions of the genome that contain genetic differences which decrease gene flow between populations. In essence, genome-wide RI is the sum of all local RI averaged across the genome. Local RI may be useful to understand the effect of selection on a genome, but it will be heavily dependent on the strength of selective sweeps, recombination rates and linkage disequilibrium. Furthermore, calculating local RI is dependent on accurately identifying causative genetic agents—a task which in and of itself is quite complicated (Lindtke & Yeaman, 2017). Thus, the effects on neutral loci near causative genetic agents will be drastically different between *Drosophila* which has linkage on the order of tens of base pairs (Mackay et al., 2012) versus maize, which has dramatically longer linkage (Rafalski & Morgante, 2004). As a result, the calculations of RI per loci in the genome need to account for biological differences that might affect migration rate (m) and m_e at every site, as well as the aggregate genome-wide effect of divergent selection.

3 | QUANTIFYING REPRODUCTIVE ISOLATION

Westram et al. (2022) argue that we should leverage genomic data to produce quantitative measures of RI. Given differences in expectations between distinct populations and scenarios involving distinct zones of hybridization, Westram et al. break down RI calculations into two separate approaches. The first of which examines two populations (which they refer to as a 2 deme model) in which gene flow is unidirectional, and the second refers to hybrid zones in which gene flow is bidirectional and determined by geographical location. The majority of prior methods, including the one proposed by Sobel and Chen (2014), focus on the potential individual and joint reduction in different processes to gene flow but do not measure the actual reduction at the genetic level. The Westram et al. approach to quantify RI differs from previous measurements in ways that are important to highlight. Sobel and Chen (2014) provided a thorough review of other mathematical equations to calculate RI, prior to producing their own method of calculating RI. Critically, these prior attempts focussed on phenotypes as an outcome, with a common focus on survival or mating successes. Sobel and Chen (2014) proposed an equation that bounds RI between

−1 (fully disassortative mating) and 1 (no gene flow). They did this using the probability of gene flow to calculate RI. Decreases in gene flow can be caused by prezygotic isolation (i.e. the frequency of heterospecific matings relative to all matings), by postzygotic isolation, or a combination of all possible mechanisms of isolation. One benefit of approaches of this nature is that estimation of different barriers to speciation, and gene flow, is theoretically straightforward, although the experimental work needed to calculate each of these barriers is not trivial. In fact, calculating these values often requires laboratory studies which might in turn be affected by experimental design (Coyne et al., 2005; Matute, 2014). However, the Westram et al. (2022) model focuses on quantifying RI of neutral loci genome-wide in a markedly different manner. Even though prior methods are valuable and provide key information on the relative contributions of the different mechanisms of isolation, one advantage to the Westram et al. definition of RI is that as new methods of calculating genetic divergence across neutral loci become available, Westram and colleagues argue that it should be feasible to calculate proxies of RI strictly with genomic data from the field. Thus, calculations of RI will become more tractable in non-model organisms, including those that are long-lived. Therefore, this new approach has the potential to facilitate comparisons across taxa that are not experimentally tractable and provide new insight into RI and speciation across the tree of life.

4 | CAVEATS OF THE MODEL

Westram and colleagues propose a novel definition of RI that merges subtly different perspectives of RI in both the organismal and the genetic contexts. This definition has the potential to be formative to the field. However, as speciation does not proceed in identical fashion in all organisms, the metrics proposed by Westram et al. (2022) are limited (as is the case for all other models as well). Several of these caveats were explicitly mentioned by the authors themselves. For example, the proposed model is only suitable for sexually reproducing organisms. Furthermore, there should be a reasonable expectation of ongoing gene flow between taxa/populations (e.g. areas of secondary contact or hybrid zones). As with other models of RI, timing collections based on species' natural history is important to prevent a disproportionate number of individuals in the data set that are unlikely to survive to maturity (e.g. in species with explosive breeding cycles). Furthermore, defining the taxa/populations of interest at which collections are done is important. Critically, expectations for individuals may be different between the 2-deme and hybrid zone scenarios. In the 2-deme model, gene flow should taper off over geographical distance barring the effect of strong ecogeographic agents. Thus, sampling two ‘populations’ in close proximity may lead to a deflated view of RI, whereas sampling too far apart may lead to the conclusion that RI is nearly complete. Unfortunately, there is no prescriptive method to discerning appropriate sampling distance, as it will depend on the strength of causative genetic agents, demography, geography/habitat differences and species traits such as vagility. The distinction Westram et al. make between the 2-deme model and hybrid zones is intuitive given the context of how differently these systems may perform in natural settings. In spite of the effort to propose a centralized framework, the resulting values of RI in the 2-deme model (RI_{2d}) and the hybrid zone models (B) have different upper bounds. RI in the 2-deme model RI_{2d} ranges from 0 (no RI) to 1 (full RI), whereas B is not bound by 1 and is instead in the units of geographical distance. Thus, making direct comparisons between taxa calculated in these two manners in a systematic way is not feasible. We note that most of these caveats apply to all studies of RI, and in no way decrease the utility of the model proposed by Westram et al.

Additionally, we believe that although their definition is promising in principle, practically applying their definition to genomic data will be challenging. Several key factors, including adequately identifying ‘causative genetic agents’ and calculations of m and m_e, are far from trivial to measure. Genomic scans (e.g. F_{ST}, Dxy, outlier tests) may identify loci that are associated with RI but they do not provide evidence of causation and are unlikely to identify all loci contributing to RI (Lindtke & Yeaman, 2017). Methods derived from examinations of polymorphisms in the site frequency spectrum for identifying loci under epistatic selection seem promising (Blanckaert & Payseur, 2021) but are also incapable of providing evidence of causation in the absence of additional testing. Similarly, calculating RI using migration rates (m) and effective migration rates (m_e) is also non-trivial. Several approaches to calculate m_e exist and all are based on estimated migration rates (Barton & Bengtsson, 1986; Beerli, 1998; Marcus et al., 2021; Yamamichi & Innan, 2012). As an additional note, if the onset of speciation or isolation is rapid (for example, via a ploidy event, novel ecogeographic barrier, or the evolution of a new phenotype which can prevent the overlap of breeding phenologies), then the calculations presented by Westram et al. (2022) are likely to be a vast under-representation of RI due to the slow evolution of neutral alleles between these populations. The approach proposed by Westram et al. has merit, and in combination with new genomic analyses to calculate several key parameters (e.g. m, m_e), this approach will represent a substantial step forward as a mechanism of unifying the field and producing measurements of RI that would be directly comparable between taxa.

5 | LOOKING TO THE FUTURE

Arguably, the greatest goal of Westram and colleagues is to provide a single, unifying calculation to examine RI. Being able to report these genome-wide (and potentially at every locus) will be an incredible boon to RI studies, particularly those that are synthetic or meta-analytical in nature. This is, of course, a problem that has long plagued the field. Indeed, their definition of RI as a quantitative measure of the flow of neutral alleles in the presence of genetic differences that reduce gene flow has a lot of promise. As they say, this definition has the potential to unite researchers from divergent research backgrounds. Furthermore, a quantitative method of calculating RI genome-wide between multiple taxa/populations would provide a dramatic boost in our ability to rapidly and systematically examine RI in natural systems.

A unified approach to measure RI also has the potential to help answer some of the most pressing questions in evolutionary biology because calculating such a metric will lend itself to better synthetic comparisons between taxa (Matute & Cooper, 2021). Extensive data compilations already exist but as authors have used different metrics of RI and genetic distance (Coughlan & Matute, 2020; Sobel & Chen, 2014), comparisons between taxa have been challenging. Some exceptions marked the way as a few studies have compared the amount of genetic divergence to attain reproductive isolation across clades (Fitzpatrick, 2002; Prager & Wilson, 1975; Wilson et al., 1974). These type of comparative assessments are sorely needed to determine whether there is a common blueprint in the way that RI accumulates and tests several prevalent hypotheses such as the relative importance of sexual isolation vs postzygotic isolation, and the prevalence of reinforcement in species that have shared geographic ranges (Matute & Cooper, 2021).

Another avenue of future research based on the Westram et al. definition of RI is to use this new definition of RI to explicitly consider speciation events within sympatry. The advantage of their definition (the flow of neutral loci given the presence of causative genetic agents that decrease gene flow) is that it is easily applicable to taxa in sympatry without obvious phenotypic differences. Thus, this definition, coupled with an analysis of RI using purely genomic data which we suggest above, could make calculations of RI in the earliest stages of speciation or divergence feasible.

6 | CONCLUSIONS

In 'What is reproductive isolation?', Westram et al. (2022) argue that a clear understanding of reproductive isolation which is universally applied by the field is critically important. The reality is that identifying the amount of RI required to identify species boundaries is a somewhat murky prospect (Roux et al., 2016). Even though a few efforts have studied how predictive genetic divergence (and phylogenetic information) can be of RI (Dettman, Jacobson, & Taylor, 2003; Dettman, Jacobson, Turner et al., 2003; Liti et al., 2006), this piecemeal approach is painstakingly slow and not suited for comparative analyses. Regardless of the actual method to measure RI and the actual scope (either genetic or organismal), Westram et al. mark the path to solve an issue that, as a field, we will need to face sooner rather than later: whether we can work under a unified set of definitions and by doing so, measure RI in a comparable way across taxa. We might also decide that we do not need a synthetic approach and that speciation processes cannot be compared across different groups. Regardless, the discussion is worth having.

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AMMS and DRM contributed to the writing of this manuscript.

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CONFLICT OF INTEREST

We declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

PEER REVIEW

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