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INVITED COMMENTARY

Reproductive isolation is a heuristic, not a measure: a commentary on Westram et al., 2022

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Abstract

Reproductive isolation is the heuristic basis of the biological species concept, but what is it? Westram et al. (this issue) propose that it is a measurable quantity, "barrier strength," that prevents gene flow among populations. However, their attempt to make the concept of reproductive isolation more scientific is unlikely to satisfy the diverse opinions of all evolutionary biologists. There are many different opinions about the nature of species, even under the biological species concept. Complete reproductive isolation, where gene flow is effectively zero, is regarded by some biologists as an important end point of speciation. Others, including Westram et al., argue for a more nuanced approach, and they also suggest that reproductive isolation may differ in different parts of the genome due to variation in genetic linkage to divergently selected loci. In contrast to both these approaches, we favour as a key criterion of speciation the stable coexistence of divergent populations in sympatry. Obviously, such populations must be reproductively isolated in some sense, but neither the fraction of the genome that is exchanged, nor measures of overall barrier strength acting on neutral variation will yield very precise predictions as to species status. Although an overall measure of reproductive isolation is virtually unattainable for these reasons, its early generation components, such as assortative mating, divergent selection, or hybrid inviability and sterility are readily measurable and remain informative. For example, we can make the prediction that to remain divergent in sympatry, almost all sexual species will require strong assortative mating, as well as some sort of ecological or intrinsic selection against hybrids and introgressed variants.

KEYWORDS
genotypic cluster, sympatry, species concept, speciation, gene flow

Reproductive isolation (RI) is viewed by many to be fundamental to the understanding of speciation; it is considered a "core concept" in evolutionary biology (Westram et al., 2022). Measures of reproductive isolation are believed to be essential for understanding and testing theories of the progress of speciation (Coyne & Orr, 2004). But what is reproductive isolation? In the traditional Dobzhansky-Mayr view of speciation, reproductive isolation consists of prezygotic isolation (for example, avoidance of mating with another group of individuals or species), and postzygotic isolation (low fitness of hybrids between groups of individuals or species). In their review, Westram et al. attempt to answer the question of what reproductive isolation is by developing an overall measure based on barriers to gene flow.

In contrast to this "core concept" view of reproductive isolation, one of us wrote: "To say that biological species are characterized by 'isolating mechanisms' is an empty statement. To include such an enormous number of different effects under a single label must be one of the most extraordinary pieces of philosophical trickery ever foisted successfully on a community of intelligent human beings. We would be far better off thinking about levels of gene flow (so-called 'prezygotic isolation') and stabilizing or disruptive selection (so-called 'postzygotic isolation') separately, rather than attempting to cope with a grab-bag term that attempts to incorporate both" (Mallet, 1995). Twenty-seven years later, biologists tend to discuss "reproductive barriers" rather than the group-selectionist-sounding term "isolating mechanisms" used before the 1990s. Today, the same author would perhaps attempt a more diplomatic tone, but the statement still conveys our view of reproductive isolation. We do not think it is very sensible or useful to quantify reproductive isolation overall; even though many components of what we mean by reproductive isolation are measurable, and are of interest in studying speciation.

We are not arguing that sexual species are not reproductively isolated; obviously, they are in some sense, or the term could not have been used by Dobzhansky and Mayr in their promotion of the biological species concept. Instead, we believe that exact measures of overall reproductive isolation do little to discover what it is we mean by species (as opposed to subspecies or varieties), or to understand how speciation occurs. Westram et al. (2022) apparently conclude similarly when they state "After reviewing methods for estimating it…, [we find] it is difficult to measure RI [reproductive isolation] in practice." Those authors then offer a few olive branches of hope: "…existing methods, especially when combined and interpreted with appropriate caution, can give insight into the extent to which populations evolve independently and the underlying barriers to gene flow. Looking to the future, we encourage researchers to explore new, creative approaches to estimating RI". We are not reassured. Reproductive isolation, in our view, is a nice heuristic although not amenable to algorithmic measurement overall. In the following pages, we explain why.

1 | HISTORY OF THE CONCEPT OF REPRODUCTIVE ISOLATION

It may help us understand how we got into this mess by reviewing the origins and history of the idea of reproductive isolation before and since Darwin. In pre-historic times, long before Darwin, it was known that a cross between horse and donkey yielded a mule. Mules were sterile, so this seemed to provide a clear distinction between species: within species, crosses were fertile. Aristotle documented this in Ancient Greece, and hybrid sterility as a concept of species was discussed in scientific publications at least by the 18th Century (Hunter, 1787). Hunter argued that dogs and wolves were members of the same species because they did not show sterility in hybrids, unlike mules. By Darwin's time, the Comte de Buffon (also cited by Hunter) was perhaps the most famous naturalist to argue for hybrid sterility as the defining characteristic of species, again using the mule as an example (Buffon, 1753).

Buffon apparently believed that hybrid sterility was a trait given by God to His created species to prevent their interbreeding. This became the prevalent view among establishment British scientists before the 1850s (Lyell, 1837). Darwin needed to disprove the creationist reproductive isolation concept to promote his alternative theory, evolution. Darwin argued that taxa recognized as good species can often be hybridized without hybrid sterility (many plants for example), and that sterility within taxa (for example, sterility among pin or thrum forms of the flowers of *Primula* species) was also common. Although Darwin agreed that hybrid sterility was loosely correlated with what we mean by species, he inferred that it accumulated as a by-product of evolution, rather than being the cause of speciation or a requirement for the existence of species. Darwin's argument explained such odd findings as asymmetries in hybrid sterility. He documented that between some pairs of plant species, a cross between A pollen and B ovules was sterile, even though crosses between B pollen and A ovules could be fertile (Darwin, 1859). If you are a supporter of a reproductive isolation concept of species, Darwin's rejection of a facet of reproductive isolation as the cause of species seems almost incomprehensible today, and this is perhaps one reason why Mayr in particular grievously misunderstood Darwin's view of species (Mallet, 2008, 2010; Mayr, 1963).

Westram et al. (2022) argue that the entomologist Edward Bagnall Poulton was maybe the first to propose "asyngamy" as a sort of reproductive isolation definition of species, and that he received some of his ideas from a monograph on *Papilio* butterflies of the Malay Archipelago by Wallace (1865). However, we believe this provides an oversimplification of the actual views of both Wallace and Poulton. Poulton, as well as Wallace and Darwin, were on the contrary arguing *against* the idea that what is now known as reproductive isolation offered a clear distinction between species. Both Wallace and Poulton appreciated Darwin's treatment of the subject, and in both cases, they rejected overall reproductive isolation as a definition of species.

The Wallace quotation used by Westram et al. is as follows: "Species are merely those strongly marked races or local forms which, when in contact, do not intermix, and when inhabiting distinct areas are generally believed to have had a separate origin, and to be incapable of producing a fertile hybrid offspring" (Wallace, 1865). This indeed seems like a reproductive isolation definition, but, like some of the passages in Darwin, it was actually just intended as a restatement of the pre-Darwinian view on sterility and creation ("separate origin") of species. In the very next (rather long) sentence, Wallace immediately negates this view: "But as the test of hybridity cannot be applied in one case in ten thousand, and even if it could be applied, would prove nothing, since it is founded on an assumption of the very question to be decided — and as the test of separate origin is in every case inapplicable — and as, further, the test of non-intermixture is useless, except in those rare cases where the most closely allied species are found inhabiting the same area, it will be evident that we have no means whatever of distinguishing so-called 'true species' from the several modes of variation here pointed out, and into which they so often pass by an insensible gradation." Briefly, Wallace here argues that there is a continuum, and there's no good place to cut it: instead of having a created "separate origin," species actually evolved gradually from one another.

As detailed by Westram et al., Poulton's argument for "asyngamy" as a definition of species was almost certainly influenced by his older friend Wallace's (1865) paper, and by *Papilio* butterflies in general. Later, Poulton's argument for the reality of species was influential in Europe, including Russia, and for Dobzhansky in particular (who was a Russian entomologist before moving to USA to work on the genetics of *Drosophila*), and Mayr (Mallet, 2004; Poulton, 1904). These ideas then coalesced as the reproductive isolation concept of species.

While this is a likely history of the idea, it should be noted that the "asyngamy" of Poulton was not the same as the reproductive isolation of Dobzhansky and Mayr. The term "syngamy" means the coming together of mating partners, and its absence between species was to Poulton the reality that underlay species. Poulton argued, following Darwin, that the evolution of hybrid sterility was an accidental result of asyngamy, and that it accumulated after speciation, rather than being an important component or cause of asyngamy itself (Poulton, 1904). It seems clear that in Dobzhansky-Mayr terms, Poulton's asyngamy was equivalent to the prezygotic component of reproductive isolation, and that postzygotic isolation was not a part of asyngamy.

2 | WHAT IS REPRODUCTIVE ISOLATION?

What do we mean by reproductive isolation? The term reproductive isolation was promoted by Dobzhansky and Mayr as a characteristic of species: members of different species did not mate together, or if they did, produced hybrids with low fitness. Individuals of opposite sex within a species are not reproductively isolated from each other, but individuals of opposite sex in different species are reproductively isolated. In the reproductive isolation world view, sexual species are real because they have a quality that populations within species do not have: reproductive isolation. Dobzhansky and Mayr fought back against the Darwinian view that species were not real (Dobzhansky, 1935, 1937; Mayr, 1963).

In their paper, Westram et al. contrast several issues about reproductive isolation:

2.1 | Qualitative or quantitative?

In its original Dobzhansky-Mayr concept, reproductive isolation was apparently a quality that species had. It prevented gene flow between species, so that species avoided being muddled together. In this conception, it is an on–off trait, not so different from the pre-Darwinian creationist viewpoint. Westram et al. instead call for a more quantitative nature of reproductive isolation. In this, they follow other workers attempting to combine various partial barriers to gene flow (Coyne & Orr, 1989). If two populations are separated by reproductive isolation (RI), the barrier to gene flow in either direction is assumed to have effect $RI = 1 - m_e/m$. Here, m (<0.5, usually) is the rate of genomic replacement expected in each receiving population in the absence of the barrier, and m_e is the effective rate achieved in the presence of the barrier that may be due to selection, assortative mating, or other impediment to gene flow. If there are three different reproductive isolation barriers (RI_1, … RI_3) that act sequentially, say ecological, assortative mating, and hybrid sterility, then the total barrier is something like:

$$RI_{tot} = 1 - (1 - RI_1)(1 - RI_2)(1 - RI_3)$$

An early elaborate calculation done in this vein was carried out with the bee-pollinated *Mimulus lewisii* and its humming-bird-pollinated sister species *Mimulus cardinalis*: nine different reproductive barriers were measured consisting of, for example, ecogeographic isolation, pollinator isolation, pollen precedence, F1 seed and seedling survivorship, F1 biomass, pollen viability, and seed mass (Ramsey et al., 2003). In *Mimulus*, prezygotic and ecological factors to do with pollination ecology were found to be more important than classical postzygotic isolation affecting intrinsic hybrid viability or sterility.

However, this still leads us back to the question: what is reproductive isolation? Prezygotic isolation, F1 hybrid sterility, and F1 viability are not the only factors that prevent gene flow. Hybrid breakdown in backcross generations, or F2 viability might be involved. Actually, we should trace gene flow through an infinite number of generations after hybridization if we are really interested in the overall barrier to gene flow between two populations (Westram et al., 2022: equation 2).

In addition, there is a simple question of which sequential barrier is most important. Suppose we have a simple system with just two barriers, prezygotic and postzygotic isolation. Suppose both barriers to gene flow are 99% effective. After the prezygotic barrier, only 1% of potential crosses between the populations produce families, and then, of these, only 1% of offspring are fertile. One way of accounting would argue that prezygotic and postzygotic isolation are equally effective, because both prevent 99% of gene flow via each m_e/m term. But a different way of accounting might argue that since postzygotic isolation acts only after prezygotic gene flow, it only has a 0.99% effect in preventing overall gene flow (i.e. a 99% reduction acting on the m = 1% of gene flow that remains after the prezygotic barrier) compared with prezygotic isolation's 99%. In nature, prezygotic barriers, particularly assortative mating, often seem more important than postzygotic isolation, suggesting that they evolve first. In an elaborate study of 12 different barriers to gene flow in the Z and E pheromone races of the European corn borer (*Ostrinia nubilalis*), prezygotic factors were most important (Dopman et al., 2010). Other empirical examples where prezygotic isolation appears to evolve early during incipient speciation are found in bimodal hybrid zones ([f] below), and in phytophagous host races in insects (Drès & Mallet, 2002). In contrast, based on simulations of some rather specific population genetic models of assortative mating and hybrid inviability, the opposite argument has been made that prezygotic isolation is much less important than postzygotic isolation, based on the long-term, rather the short-term effects of gene flow (Irwin, 2021).

So which is the most important source of reproductive isolation? It partly seems a matter of personal choice and focus on different measures of speciation rather than any real decision based on numbers.

And it is worse than that. Dobzhansky included geographic isolation in his list of "isolating mechanisms," while Mayr excluded it (Dobzhansky, 1937; Mayr, 1963). In support of Dobzhansky, if a reproductive barrier has a quantitative effect on prevention of gene flow, the barriers should include spatial separation between taxa because it does affect "gene flow in the absence of a reproductive barrier, *m*." Westram et al. also refer to a geographic element to reproductive isolation when they discuss the effect of geographic barriers to gene flow in hybrid zones. But to Mayr (and to Westram et al.), geography was not a genetically encoded property of the species, and so should be excluded from reproductive isolation (Mayr, 1963). We think most would today side with Mayr and Westram et al.; however, the "ecogeographic isolation" already discussed in *Mimulus* certainly includes partial geographic separation (Ramsey et al., 2003).

A related issue is immigrant inviability (Nosil et al., 2005). Supposing a pair of sister species utilizes different host plants. When an insect of one of the species migrates to the host plant of the other, it may suffer immediate loss of fitness due to lack of adaptation to the new host; this will reduce gene flow even before reproduction, and therefore, it hardly qualifies as "reproductive" isolation; it is more importantly divergent selection, which can act both before and after reproduction. Yet, this selection can be very important in reducing gene flow. Westram et al., perhaps for this reason, include immigrant inviability within their concept of reproductive isolation.

In diploids, F1 hybrid sterility and inviability of a given strength acts against a single genome of each species, and so have half the effect of reducing gene flow from one species to another as the same reduction of flow due to assortative mating, which prevents an entire diploid individual from "migrating" to another species (Kirkpatrick & Ravigné, 2002). Immigrant inviability also has this stronger effect of acting against foreign diploids compared with postzygotic unfitness of hybrids, which affects only a haploid genome.

Most of these thoughts, however, deal only with the earlier generations of hybridization and introgression. Getting back to the point raised earlier about needing to account for an infinite number of generations after hybridization, when we consider gene flow from one population to another, Westram et al. show (following Bengtsson, 1974) that the overall "reproductive isolation" when preventing neutral gene flow is approximately $RI_{tot} = 1—\overline{W}_0\overline{W}_1\overline{W}_2^{\,2}$, where $\overline{W}_0$ includes the average probability of assortative mating as well as the average immigrant viability of migrants, $\overline{W}_1$ is a measure of the average F1 hybrid viability and fertility, and $\overline{W}_2$ is the average fitness of first generation backcross hybrids; the squared backcross term is explained because each further generation of backcrossing $(\overline{W}_3, \overline{W}_4, \overline{W}_5, \ldots)$, assuming hybrids are rare, dilutes the average prevention of gene flow by a factor of ~½.

In conclusion, measurement of reproductive isolation as a quantity is complicated, in part because it depends on what we intend by reproductive isolation, and because different people have different ideas and use the "language of speciation" differently (Harrison, 2012).

2.2 | Is reproductive isolation measured at neutral loci or divergently selected loci?

We were surprised that Westram et al. propose to measure reproductive isolation not at the loci that produce reproductive isolation, but instead only at other loci in the genome that have no effect on fitness, neutral loci (see also Barton & Hewitt (1985)). In all fairness, they have a good argument that if barriers prevent gene flow at neutral loci, they also prevent it at selected loci as well. Nonetheless, it seems odd that the loci causing reproductive isolation cannot be used themselves to gauge the strength of reproductive isolation. Because postzygotic isolation loci, at least, are usually disfavoured in other species, it would be more direct to study the selection itself, rather than try to first infer their effect on overall reproductive isolation before investigating whether these loci cause speciation (Mullen & Shaw, 2014; Shaw & Mullen, 2011).

Originally, this approach seemed justified because it allowed geographic populations differing clinally only at a few selected loci to be classified as members of the same species; they were not reproductively isolated in the sense that neutral gene flow can still take place. However, this view is also associated with the idea that speciation is complete only when gene flow is completely absent (Barton & Hewitt, 1985). This method of measuring reproductive isolation can lead to difficulties with sympatric, hybridizing species. For example, *Anopheles gambiae* and *A. coluzzii* are known to differ in sympatry at only a few "islands of speciation" in the genome (see also discussion of "islands of speciation" in [2.5] below). The divergent islands form in only ~1% of the genome, and the rest of the genome is virtually homogenized by occasional hybridization and gene flow. Once they have recombined away from strongly selected loci, there is little prevention of neutral variants from crossing between the two species (Turner et al., 2005). Nonetheless, these two species are sympatric over a vast area of tropical Africa, and otherwise behave like "good species", with differences in ecology and strong assortative mating in the field; they are reproductively isolated enough to be recognized as separate species, and yet, hybridize enough to homogenize neutral genomic variation (Lee et al., 2013).

2.3 | Gene flow between two populations, or gene flow across a hybrid zone?

Westram et al. discuss how measuring gene flow between two populations (or species, perhaps) as detailed above is not the only situation where we might measure reproductive isolation. A hybrid zone between two spatially distributed populations differing at multiple loci will create a barrier to gene flow that can also be characterized as a kind of reproductive isolation. The gene flow barrier under one approximation is equivalent to an extra (imaginary) distance over which genes must flow to get to the other side of the hybrid zone (Barton & Bengtsson, 1986). A physical barrier (such as a region unsuitable for either population) will also create a barrier to gene flow, and there is no easy way to distinguish the two from genomic data (Westram et al., 2022).

As Westram et al. clearly point out, understanding reproductive isolation via gene flow across a narrow hybrid zone in space is rather different than understanding it via gene flow between two, say, sympatric species. In the spatial hybrid zone model, mating may be entirely random in all local populations, and the local situation in the cline can be viewed, therefore, as crossing between divergent genetic individuals within a species. The barrier strength in terms of overall prevention of gene flow measured between a pair of sympatric species, in contrast, could be similar. Yet, the mating behaviour is highly non-random in most real cases of sympatric species. In one view of species, the two-population situation represents reproductive isolation between a pair of sympatric species, while the cline model represents a model of divergent selection. In Mayr's view, the kinds of selection leading to clines were not included in reproductive isolation (Mayr, 1963). Mayr promoted his “biological species concept” that classified parapatric taxa across hybrid zones and clines as members of the same species, often as subspecies.

An alternative opinion is that if the prevention of gene flow is what we mean by reproductive isolation, both situations would be awarded equivalent status when they had the same levels of overall barrier strength measured. This seems to be the meaning of reproductive isolation of Westram et al. This kind of thinking has more recently permeated systematics, even by those who nominally support a biological species concept. Many parapatric and hybridizing North American bird taxa, classified as subspecies under Mayr's version of the biological species concept, have recently been re-elevated to full species status, because clinal selection is being re-interpreted as part of reproductive isolation (Gill, 2014).

As Westram et al. point out, two-deme reproductive isolation (e.g. between sympatric populations) and clinal reproductive isolation via barrier strengths are not directly comparable. Although both have effects on gene flow, they do not map onto what everyone would agree we need to measure in order to understand speciation. In our own opinion, stable coexistence in sympatry is the hallmark of speciation; in sympatry, if you like, there must be sufficient reproductive isolation to allow species to coexist. Divergence may be swamped by gene flow at some loci, but in order for the populations to be regarded as species rather than mere polymorphisms, there must be divergence at multiple loci across the genome characterized by strong linkage disequilibrium among them (Felsenstein, 1981; Mallet, 1995; Mallet et al., 2022).

2.4 | “Organismal” or “genetic aspects” of reproductive isolation?

Westram et al. also highlight another distinction: organismal versus genetic aspects of reproductive isolation. We found this distinction hard to follow, but if we understand correctly “organismal” refers to the production or fitness of whole organisms, particularly early generation hybrids, whereas the “genetic” aspect refers to the long-term effect on gene flow, which may affect only parts of the genome. Thus, the organismal focus is referring to the first few generations of hybridization, whereas the genetic focus is about gene flow effects over all generations of actual or potential hybridization; it consists of the infinite series of terms in $1 - m_e/m$. In any case, Westram et al. come clearly down on the side of the genetic focus, but also “encourage the use of methods for estimating reproductive isolation that integrate the organismal and genetic approaches.”

2.5 | Whole genome or individual locus reproductive isolation?

In its original organismal focus, reproductive isolation was something that applied to whole genomes, and to the organisms that carry them. However, given that gene flow is possible, and does seem to take place between the taxa we call species, and given that not all genes are permitted to flow, the genome will contain some loci that are divergently selected, and other loci that can flow between species. Our work on differential gene flow in parts of the genome of pairs of species led one of us to formulate an alternative species definition, the genotypic cluster definition that focused on the parts of the genome that were stable to gene flow and remained differentiated (Mallet, 1995).

Similarly, Chung-I Wu in his genic view of speciation argued that different parts of the genome could in essence differ in levels of reproductive isolation, and that this was not covered by the classical biological species concept (Wu, 2001). Later, studies of sympatric populations of *Anopheles gambiae* and its sibling species *A. coluzzii* (then known as the M and S races of *A. gambiae*) yielded evidence that divergence between the two species was concentrated at a few islands of divergence that formed a small portion of the overall genome (Lee et al., 2013; Turner et al., 2005). A controversy arose about the idea that stable divergence could be achieved at only some loci, while other loci flowed relatively freely between species. To some, this seemed a rather unlikely population structure, and previously documented cases were argued to be artefacts of incorrect data analysis (Cruickshank & Hahn, 2014). However, it is clear that this kind of population structure can nonetheless be a real phenomenon in nature, for example, in the hybrid overlap between the carrion crow and the hooded crow (Knief et al., 2019; Metzler et al., 2021; Poelstra et al., 2014).

Westram et al., in their genetic focus (see [2.4] above) on gene flow, argue that neutral loci do indeed experience different levels of reproductive isolation across the genome, depending on physical distance and linkage to the nearest divergently selected locus. In this formulation, recombination rate of any neutral indicator locus with these selected loci will affect the levels of reproductive isolation differently across the genome, as well as levels of selection and overall gene flow.

At a single locus, Haldane showed that with gene flow between a pair of populations, if $m/s \ll 1$, then the populations could diverge, where m is the fraction of the population exchanged per generation, and s is the divergent selection coefficient against immigrants (Haldane, 1930). In a realistic genome, however, the recombination rate between strongly and weakly selected loci will affect this result: a neutral mutation may experience indirect selection due to linkage with a divergently selected locus. Nonetheless, if few loci are under direct selection, many other loci may be unlinked or weakly linked and able to flow freely between populations because for those loci $m/s > 1$. Haldane's theory explains simply why islands of divergence, and the low-divergence regions in the rest of the genome, may exist.

Models of the slow mutational accumulation of divergently selected loci between populations between which gene flow occurs suggest that divergent selection may yield transitions from single effectively panmictic populations, through pairs of populations (species) with genomic islands of divergence, which later results in a final stage where even neutral variants no longer pass readily between the populations (Rafajlović et al., 2016; Riesch et al., 2017; Schilling et al., 2018). Empirical evidence for these state transitions during and after speciation have been found, for example, in *Heliconius* butterflies (Kronforst et al., 2013; Martin et al., 2013).

2.6 | Experimental measures using crosses or inference from static genetic patterns?

Some have argued that one can detect reproductive isolation from population genetic analyses, for example, in the case of islands of speciation (see [2.5] above), or in bimodal hybrid zones (Gay et al., 2008; Harrison & Bogdanowicz, 1997; Jiggins & Mallet, 2000). A bimodal hybrid zone is one where, in areas of hybridization and sympatry, two distinct modes in the phenotypic and genotypic distribution of individuals (for example, using a hybrid index) are found. A more detailed study of this potential situation investigates individuals on a genome-wide heterozygosity index, as well as a hybrid index (Fitzpatrick, 2012).

Many genetic patterns such as overall levels of genetic differentiation, or hybrid inviability vary strongly between different bimodal and unimodal hybrid zones, but are not very predictive of bimodality. In contrast, bimodal hybrid zones show a marked tendency towards greater assortative mating than “unimodal” hybrid zones, where only a single mode in the distribution in any local population is found. In the bimodal zones, the two taxa form separate genotypic clusters in spite of some hybridization and gene flow, and therefore, it was argued that ecological factors and assortative mating (roughly identifiable as prezygotic isolation) were perhaps more important in bimodality and speciation than intrinsic postzygotic isolation (Jiggins & Mallet, 2000). Nonetheless, Westram et al. correctly caution that their focus on gene flow in measures of overall reproductive isolation does not map well onto patterns in the genomes of hybridizing taxa, such as bimodality or genetic divergence across different parts of the genome.

3 | CONCLUSIONS

Given the focus of many speciation biologists on the biological species concept, it has generally seemed that we should study reproductive isolation to understand speciation. But exactly how we should do this depends to some extent on what it is we mean by speciation, and species. Some still argue that the “completion” of speciation, and therefore we suppose “good species,” occur only when reproductive isolation is complete, and gene flow ceases altogether (Kulmuni et al., 2020; Wang et al., 2019). However, it would be very hard to use such a species concept or criterion for speciation in practice given the abundant evidence for continued gene flow between the actual taxa we call species (Barton, 2020; Mallet, 2020). In practice, therefore, speciation biologists tend to “… depart from the hard line BSC by recognizing species that have limited gene exchange with sympatric relatives” (Coyne & Orr, 2004: 30). This has the benefit of focusing on speciation as a process, rather than species status. But it also makes it unclear just which part of that process is important in speciation and does not clearly outline how we should use an overall measure of reproductive isolation in studying speciation.

Reproductive isolation is a useful heuristic in understanding the nature of species. However, when it comes to measuring its components and putting them together in an overall measure of reproductive isolation, what that means becomes rather unclear. There are almost as many different measures of overall reproductive isolation as there are authors who have commented on the problem, well shown in two reviews (Sobel & Chen, 2014; Westram et al., 2022). This likely demonstrates not just that overall reproductive isolation, like the species to which this property belongs, are difficult concepts, but also that neither are “real” in the sense of having discrete boundaries.

In contrast, various components of reproductive isolation, such as assortative mating, disruptive selection, and F1 hybrid inviability or sterility, have a clear meaning, are readily measurable, and will remain useful in studying speciation, even though their relative importance will differ according to context. For example, careful reanalysis of laboratory crosses in *Drosophila* (Coyne & Orr, 1989) tested whether the strength of assortative mating (prezygotic isolation) was related to the strength of hybrid inviability and sterility (postzygotic isolation) between sympatric species. If correlated, this would add further evidence for the original finding of reinforcement in sympatry, with stronger postzygotic selection leading to stronger selection for assortative mating (Turelli et al., 2014). As it turned out, no significant correlation between the two measures of reproductive isolation was found. This did not necessarily negate the likelihood of reinforcement, as the authors point out: the lack of the expected correlation is likely because there are many components of postzygotic isolation not revealed by the lab experiments, such as unmeasured ecological factors (Turelli et al., 2014). Our point here is not that the approach was particularly successful, but that it did represent good use of the data on components of reproductive isolation at hand.

Westram et al. make a laudable attempt towards a unified measure of reproductive isolation. Here, we argue instead that overall reproductive isolation is perhaps best left as a whole-organism heuristic related to the biological species concept. Nonetheless, individual components of what we might call reproductive isolation, such as assortative mating, disruptive selection, and hybrid inviability, remain important for understanding the effects on gene flow in specific cases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Barton, N. H. (2020). On the completion of speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*, 20190530.

Barton, N. H., & Bengtsson, B. O. (1986). The barrier to genetic exchange between hybridizing populations. *Heredity*, *57*, 357–376.

Barton, N. H., & Hewitt, G. M. (1985). Analysis of hybrid zones. *Annual Review of Ecology and Systematics*, *16*, 113–148.

Bengtsson, B. O. (1974). *Karyotype evolution in vivo and in vitro*. PhD Thesis. University of Oxford.

Buffon, G.-L. L. (1753). Description de la partie du Cabinet qui a rapport à l'histoire naturelle du cheval. L'asne. In *Histoire Naturelle, Générale et Particuliére, avec la Description du Cabinet du Roy* (Vol. *4*, pp. 377–403). Imprimerie Royale.

Coyne, J. A., & Orr, H. A. (1989). Patterns of speciation in *Drosophila*. *Evolution*, *43*, 362–381.

Coyne, J. A., & Orr, H. A. (2004). *Speciation*. Sinauer Associates.

Cruickshank, T. E., & Hahn, M. W. (2014). Reanalysis suggests that genomic islands of speciation are due to reduced diversity, not reduced gene flow. *Molecular Ecology*, *23*, 3133–3157.

Darwin, C. R. (1859). *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray.

Dobzhansky, T. (1935). A critique of the species concept in biology. *Philosophy of Science*, *2*, 344–355.

Dobzhansky, T. (1937). *Genetics and the origin of species*. Columbia University Press.

Dopman, E. B., Robbins, P. S., & Seaman, A. (2010). Components of reproductive isolation between North American pheromone strains of the European corn borer. *Evolution*, *64*, 881–902.

Drès, M., & Mallet, J. (2002). Host races in plant-feeding insects and their importance in sympatric speciation. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, *357*, 471–492.

Felsenstein, J. (1981). Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution*, *35*, 124–138.

Fitzpatrick, B. (2012). Estimating ancestry and heterozygosity of hybrids using molecular markers. *BMC Evolutionary Biology*, *12*, 131.

Gay, L., Crochet, P. A., Bell, D. A., & Lenormand, T. (2008). Comparing clines on molecular and phenotypic traits in hybrid zones: A window on tension zone models. *Evolution*, *62*, 2789–2806.

Gill, F. B. (2014). Species taxonomy of birds: Which null hypothesis? *The Auk*, *131*, 150–161.

Haldane, J. B. S. (1930). A mathematical theory of natural and artificial selection. 6. Isolation. *Proceedings of the Cambridge Philosophical Society*, *26*, 220–230.

Harrison, R. G. (2012). The language of speciation. *Evolution*, *66*, 3643–3657.

Harrison, R. G., & Bogdanowicz, S. M. (1997). Patterns of variation and linkage disequilibrium in a field cricket hybrid zone. *Evolution*, *51*, 493–505.

Hunter, J. (1787). Observations tending to shew that the wolf, jackal, and dog, are all of the same species. *Philosophical Transactions of the Royal Society of London*, *77*, 253–266.

Irwin, D. (2021). Assortative mating in hybrid zones is remarkably ineffective in promoting speciation. *The American Naturalist*, *195*, E150–E157.

Jiggins, C. D., & Mallet, J. (2000). Bimodal hybrid zones and speciation. *Trends in Ecology and Evolution*, *15*, 250–255.

Kirkpatrick, M., & Ravigné, V. (2002). Speciation by natural and sexual selection. *American Naturalist*, *159*, S22–S35.

Knief, U., Bossu, C. M., Saino, N., Hansson, B., Poelstra, J., Vijay, N., Weissensteiner, M., & Wolf, J. B. W. (2019). Epistatic mutations under divergent selection govern phenotypic variation in the crow hybrid zone. *Nature Ecology & Evolution*, *3*, 570–576.

Kronforst, M. R., Hansen, M. E. B., Crawford, N. G., Gallant, J. R., Zhang, W., Kulathinal, R. J., Kapan, D. D., & Mullen, S. P. (2013). Hybridization reveals the evolving genomic architecture of speciation. *Cell Reports*, *5*, 666–677.

Kulmuni, J., Butlin, R. K., Lucek, K., Savolainen, V., & Westram, A. M. (2020). Towards the completion of speciation: The evolution of reproductive isolation beyond the first barriers. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*, 20190528.

Lee, Y., Marsden, C. D., Norris, L. C., Collier, T. C., Main, B. J., Fofana, A., Cornel, A. J., & Lanzaro, G. C. (2013). Spatiotemporal dynamics of gene flow and hybrid fitness between the M and S forms of the malaria mosquito, *Anopheles gambiae*. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 19854–19859.

Lyell, C. 1837. *Principles of geology, being an inquiry how far the former changes of the earth's surface are referable to causes now in operation* (Vol. *2*, 5th edn). John Murray.

Mallet, J. (1995). A species definition for the modern synthesis. *Trends in Ecology and Evolution*, *10*, 294–299.

Mallet, J. (2004). Poulton, Wallace and Jordan: How discoveries in *Papilio* butterflies initiated a new species concept 100 years ago. *Systematics and Biodiversity*, *1*, 441–452.

Mallet, J. (2008). Mayr's view of Darwin: Was Darwin wrong about speciation? *Biological Journal of the Linnean Society*, *95*, 3–16.

Mallet, J. (2010). Why was Darwin's view of species rejected by 20th Century biologists? *Biology and Philosophy*, *25*, 497–527.

Mallet, J. (2020). Alternative views of biological species: Reproductively isolated units or genotypic clusters? *National Science Review*, *8*, 1401–1407.

Mallet, J., Seixas, F., & Thawornwattana, Y. (2022). Species, concepts of. In *Encyclopedia of biodiversity*, Encyclopedia of biodiversity. 3rd Ed. Reference module in life sciences (pp. 1–15). Elsevier.

Martin, S. H., Dasmahapatra, K. K., Nadeau, N. J., Salazar, C., Walters, J. R., Simpson, F., Blaxter, M., Manica, A., Mallet, J., & Jiggins, C.

D. (2013). Genome-wide evidence for speciation with gene flow in *Heliconius* butterflies. *Genome Research*, *23*, 1817–1828.

Mayr, E. (1963). *Animal species and evolution*. Harvard University Press.

Metzler, D., Knief, U., Penalba, J. V., & Wolf, J. B. W. (2021). Assortative mating and epistatic mating-trait architecture induce complex movement of the crow hybrid zone. *Evolution*, *75*, 3154–3174.

Mullen, S. P., & Shaw, K. L. (2014). Insect speciation rules: Unifying concepts in speciation research. *Annual Review of Entomology*, *59*, 339–361.

Nosil, P., Vines, T. H., & Funk, D. J. (2005). Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, *59*, 705–719.

Poelstra, J. W., Vijay, N., Bossu, C. M., Lantz, H., Ryll, B., Müller, I., Baglione, V., Unneberg, P., Wikelski, M., Grabherr, M. G., & Wolf, J. B. W. (2014). The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. *Science*, *344*, 1410–1414.

Poulton, E. B. (1904). What is a species? *Proceedings of the Entomological Society of London*, *1903*, lxxvii–cxvi.

Rafajlović, M., Emanuelsson, A., Johannesson, K., Butlin, R. K., & Mehlig, B. (2016). A universal mechanism generating clusters of differentiated loci during divergence-with-migration. *Evolution*, *70*, 1609–1621.

Ramsey, J., Bradshaw, H. D., & Schemske, D. W. (2003). Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution*, *57*, 1520–1534.

Riesch, R., Muschick, M., Lindtke, D., Villoutreix, R., Comeault, A. A., Farkas, T. E., Lucek, K., Hellen, E., Soria-Carrasco, V., Dennis, S. R., de Carvalho, C. F., Safran, R. J., Sandoval, C. P., Feder, J., Gries, R., Crespi, B. J., Gries, G., Gompert, Z., & Nosil, P. (2017). Transitions between phases of genomic differentiation during stick-insect speciation. *Nature Ecology & Evolution*, *1*, 0082.

Schilling, M. P., Mullen, S. P., Kronforst, M., Safran, R. J., Nosil, P., Feder, J. L., Gompert, Z., & Flaxman, S. M. (2018). Transitions from single- to multi-locus processes during speciation with gene flow. *Genes*, *9*, 274.

Shaw, K. L., & Mullen, S. P. (2011). Genes versus phenotypes in the study of speciation. *Genetica*, *139*, 649–661.

Sobel, J. M., & Chen, G. F. (2014). Unification of methods for estimating the strength of reproductive isolation. *Evolution*, *68*, 1511–1522.

Turelli, M., Lipkowitz, J. R., & Brandvain, Y. (2014). On the Coyne and Orr-igin of species: Effects of intrinsic postzygotic isolation, ecological differentiation, X chromosome size, and sympatry on *Drosophila* speciation. *Evolution*, *68*, 1176–1187.

Turner, T. L., Hahn, M. W., & Nuzhdin, S. V. (2005). Genomic islands of speciation in *Anopheles gambiae*. *PLoS Biology*, *3*, e285.

Wallace, A. R. (1865). On the phenomena of variation and geographical distribution as illustrated by the Papilionidae of the Malayan region. *Transactions of the Linnean Society of London*, *25*, 1–71.

Wang, X., He, Z., Shi, S., & Wu, C.-I. (2019). Genes and speciation: Is it time to abandon the biological species concept? *National Science Review*, *7*, 1387–1397.

Westram, A. M., Stankowski, S., Surendranadh, P., & Barton, N. (2022). What is reproductive isolation? *Journal of Evolutionary Biology*.

Wu, C. I. (2001). The genic view of the process of speciation. *Journal of Evolutionary Biology*, *14*, 851–865.

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