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Genomic Approaches Are Improving Taxonomic Representation in Genetic Studies of Speciation

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Until recently, our understanding of the genetics of speciation was limited to a narrow group of model species with a specific set of characteristics that made genetic analysis feasible. Rapidly advancing genomic technologies are eliminating many of the distinctions between laboratory and natural systems. In light of these genomic developments, we review the history of speciation genetics, advances that have been gleaned from model and non-model organisms, the current state of the field, and prospects for broadening the diversity of taxa included in future studies. Responses to a survey of speciation scientists across the world reveal the ongoing division between the types of questions that are addressed in model and non-model organisms. To bridge this gap, we suggest integrating genetic studies from model systems that can be reared in the laboratory or greenhouse with genomic studies in related non-models where extensive ecological knowledge exists.

Biological speciation results from the accumulation of genetic differences that reduce gene flow between populations. This reproductive isolation evolves between lineages via mechanisms that promote assortative mating and/or cause inviability or sterility in hybrids. A longstanding goal of speciation research is to identify the genetic basis of reproductive isolation across the tree of life. Until recently, achieving this goal has been limited to a narrow set of taxa that had genetic and genomic resources available. Even then, our understanding of the genetics of speciation often remained limited to describing broad patterns of the genetic architecture of reproductive isolation, that is, the number of loci involved, their linkage relationships, and their effect sizes. However, the ultimate goal of speciation genetics is to identify specific loci and al-

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leles that initially create barriers to gene flow (e.g., chromosomal rearrangements, or genes involved in reproduction, mate choice, or hybrid fitness) along with conditions responsible for their evolution.

Early studies that focused on identifying the genetic architecture of reproductive isolation used what we here refer to as "classical" genetic techniques, such as making crosses and phenotyping or karyotyping hybrid offspring. These constraints created a historical bias in the diversity of taxa used to study speciation genetics, limiting our ability to identify general patterns. Modern genomic approaches are rapidly expanding the study of speciation genetics to a much broader set of organisms.

Here, we review the contributions to speciation genetic research made using classical genetic approaches in model systems, and we describe how genomic approaches are expanding our understanding of molecular mechanisms of speciation in more diverse species and systems. We then discuss future opportunities in speciation research that can leverage genome-scale data to further redress the historical contingencies limiting our exploration of reproductive isolation in diverse taxa.

A BRIEF HISTORY OF CLASSICAL SPECIATION GENETICS RESEARCH

Early speciation biologists recognized that characterizing barriers to genetic exchange between diverging lineages can bring us closer to understanding the origins of biodiversity (Mayr 1942; Coyne and Orr 2004). Initial studies in speciation genetics focused on systems with a few key characteristics. For example, classical genetics requires the ability to make crosses, rear large numbers of progeny in a controlled environment, track visible mutants, and employ cytogenetic techniques like chromosome squashes. In the pre-genomic era, *Drosophila* research dominated studies of speciation genetics in animals, where numerous traits under the control of complex genetic architectures were found to contribute to reproductive isolation (reviewed in Coyne and Orr 1998). For example, Sturtevant's initial (Sturtevant 1920) report of F1 hybrid sterility and inviability between *Drosophila melanogaster* and *Drosophila simulans* was later paired with cytogenetics and deficiency mapping to associate hybrid male sterility with a recessive deletion on the fourth chromosome (Muller and Pontecorvo 1940, 1942; Pontecorvo 1943). These early genetic approaches were later adopted for use in speciation research in other taxa. For example, before genome sequences were available, some of the first hybrid inviability and sterility genes identified in vertebrates were found in *Xiphophorus* fishes, using restriction fragment length polymorphism mapping, cloning, and Sanger sequencing (Wittbrodt et al. 1989). In mice, hybrid male sterility was mapped to the X chromosome using Southern analysis of DNA probes on the nuclear genomes of F1 hybrids (Guénet et al. 1990).

Biologists also capitalized on the powerful potential of plants for studies of reproductive isolation in the pre-genomic era. The Biosystematists, an organization formed in California in 1936 and dominated by botanists from the Carnegie Institution of Washington (CIW) at Stanford, UC Berkeley, UC Davis, and the California Academy of Sciences, facilitated an interdisciplinary approach to study the genetics, ecology, physiology, and paleontology of many species radiations (Smocovitis 1994, 1997). Because of the ease with which plants could be cultivated, crossed, and cytogenetically characterized, barriers to gene flow were studied in many plant groups. For example, crossing barriers were summarized for over 200 interspecific combinations of *Clarkia* in California (Lewis and Lewis 1955). Additional plant studies investigated the genetics of ecological differentiation critical to speciation. The CIW researchers used field transplants of parentals and recombinant hybrids to dissect the genetic architecture of ecogeographic and floral divergence and to measure the strength of selection on particular traits and hybrid combinations (e.g., Clausen et al. 1940, 1945; Clausen and Hiesey 1958; Hiesey et al. 1971). These classic studies demonstrated that adaptations to the local environment, both abiotic and biotic, could serve as strong isolating barriers. While much of this adaptation was

polygenic and not easily characterized, later studies with molecular markers capitalized on these early findings. For example, chromosomal rearrangements preventing gene flow were genetically mapped in sunflowers using random amplified polymorphic DNA (RAPD) markers and backcross hybrids (Rieseberg et al. 1995). Also, monkey flowers (*Mimulus*) were easily grown, widely interfertile, and exhibited striking phenotypes (McMinn 1951; Hiesey et al. 1971; Vickery 1978). Once molecular markers were developed, monkeyflowers were used in one of the earliest applications of quantitative trait locus (QTL) analysis to study reproductive isolation. Specifically, floral differences involved in prezygotic isolation were genetically mapped and tested under field conditions (Bradshaw et al. 1995, 1998; Schemske and Bradshaw 1999), rendering them one of the major model systems for speciation research (for review, see Twyford et al. 2015).

An emergent theme from pre-genomic studies is that historical contingencies have propelled certain taxa to the forefront of speciation genetics research. In particular, species with convenient traits that define genetic model organisms, and with long-standing research communities where genetic resources are developed and shared, have dominated the analysis of the genetic and molecular details of speciation (Box 1). Even after molecular markers became available for many species, genetic mapping still required segregating lines with hundreds of offspring. This limitation restricted investigation to systems amenable to cultivation and crossing and those having high fecundity, short generation times, and easily characterized phenotypes. Thus, prior to the genomic era, the catalog of known speciation genes was populated by studies in yeast, *Arabidopsis*, *Drosophila*, mouse, rice, and platyfish (Coyne and Orr 1998; Presgraves 2010; Maheshwari and Barbash 2011). Although these studies facilitated a more complete understanding of the genetic basis of species differences, they were limited by the resources available at the time. The recent development of genomic technologies has afforded new opportunities to advance our understanding of speciation genetics in eukaryotes, both in these "classic" systems as well as in additional taxa.

CONTEMPORARY SPECIATION RESEARCH IN THE GENOMIC ERA

Compared to classical speciation genetics, genomic technologies have enabled unprecedented access to new data and approaches in the last two decades, including in traditionally non-model systems (Box 1). Next-generation sequencing (NGS) includes reduced-representation sequencing, where sequencing occurs adjacent to restriction enzyme cut sites, and whole-genome resequencing. These techniques allow the simultaneous discovery and scoring of genetic variation, making them accessible to a diverse set of organisms (Davey et al. 2011; Andrews et al. 2016). In some cases, NGS uses the same study designs from past work on speciation genetics. For example, QTL analyses are still widely used to study the genetic architecture of phenotypic traits involved in speciation. The main difference is that NGS allows genetic variation to be quantified at far more markers without a priori design. This provides vastly greater genomic resolution in a larger diversity of organisms. While researchers continue to apply these tools for QTL mapping in traditional model organisms, the tools have also enabled QTL mapping in systems like salmon, stickleback fish, spruce trees, and tropical rainforest herbs (Gonen et al. 2014; Glazer et al. 2015; Fuentes-Utrilla et al. 2017; Kay and Surget-Groba 2022). Despite these advances, many of the same limitations of earlier QTL analyses still apply. For example, crosses must still be generated in taxa that can easily produce large numbers of offspring. Nonetheless, as detailed below, genomic tools have also facilitated new "top-down" and "bottom-up" approaches for exploring speciation.

"Top-Down" Mapping Approaches

Genetic mapping (including QTL and admixture mapping) is considered a "top-down" approach, whereby researchers begin with knowledge of the phenotypic traits involved in speciation and look for genetic variants under-

BOX 1. SURVEYING THE CHARACTERISTICS OF TAXA USED FOR SPECIATION GENETICS/GENOMICS RESEARCH

To illustrate taxonomic representation in speciation genetics studies, and where potential gaps remain, we broadly distributed a survey (see Supplemental File S1) to active speciation genetics/genomics scientists around the world (see Supplemental Figs. S1–S3 for summary demographics of the respondents). In part, we solicited information about the species or systems that each scientist uses (summarized in Supplemental Fig. S4). We then asked the participants to numerically rate the species or system they study on 10-point scales according to "Where does your species/system exist on the continuum from being lab-adapted/domesticated" (which we further defined as "studied exclusively in a laboratory, greenhouse, or artificial setting") to being a "natural/wild population that is studied exclusively in nature?" (Supplemental Fig. S5) and "Compared to all of the various genetic/genomic resources and tools that exist for any species/system, how many are currently available for use in your species/system?" (Supplemental Fig. S6). Figure 1 shows the distribution of the responses involving biological systems (i.e., excluding computational modeling) by taxonomic category on these axes: resource availability and the extent to which experimental work must be performed in a laboratory or greenhouse environment. Eighty-nine different systems (species, genera, or higher-order groups) were represented (Fig. 1). Scientists generally responded that many "wild" species (requiring study in a natural environment) have fewer genetic or genomic resources and tools (lower-left quadrant, including many of the vertebrate and plant species) than the animal and fungal species in the upper-right

continued

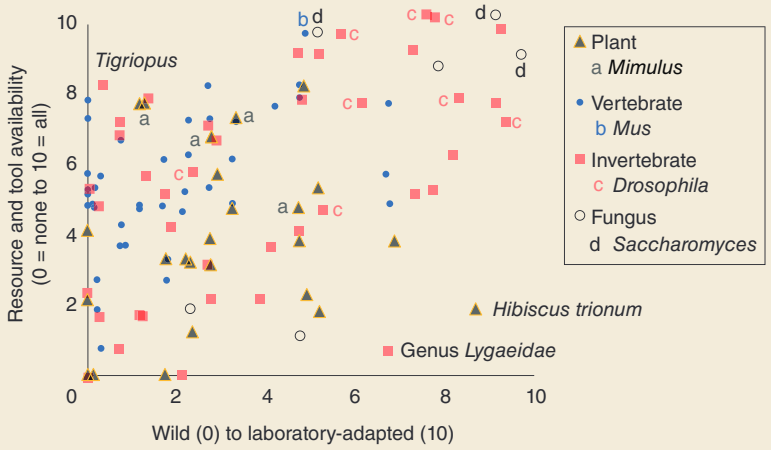

Figure 1. Taxonomic distribution of a sample of speciation genetics species and systems. Survey respondents identified the location on this Cartesian plane of 112 species or systems they study. Data points have been characterized into four taxonomic groups (plant, vertebrate, invertebrate, and fungus) and jittered on both axes to facilitate visualization of otherwise overlapping symbols. All responses for four genetic model systems (*Mus*, *Drosophila*, *Caenorhabditis*, and *Saccharomyces*) are indicated, as well as three taxa that occupy extremes of the plane: copepods in the *Tigriopus* genus (wild populations studied and have many resources and tools) and true bugs of the genus *Lygaeidae* and *Hibiscus trionum* (laboratory-rearable but resource- and tool-poor). In general, vertebrates tend to be studied in natural environments and are resource-rich, while plant researchers often report that their natural study systems have fewer available resources. Invertebrates span much of the dimensional space, and fungi are reported to be either laboratory models with many resources or wild systems with few resources. An opportunity for future work is to better develop the *upper-left* quadrant to generate more resources and tools for working with natural populations. Details of the genetic and genomic resources and tools that the respondents use are provided in Supplemental Figures S6 and S7.

quadrant, which are reared in an artificial environment. Generally, traditional genetic model systems tend to be resource-rich and studied in artificial environments (Fig. 1). The research resources and tools used by respondents are summarized in Supplemental Figure S7.

Researchers also rated various characteristics of their study system or species that facilitated investigation of speciation genetics. To identify characteristics of species or systems that have been useful for the study of speciation genetics/genomics in natural and artificial settings, we correlated these characteristics with respondents' opinions on the extent to which that system or species is reared in an artificial environment (Supplemental Table S1). Thirteen of 21 characteristics, such as low ploidy and the geographical accessibility of natural populations, were not significantly correlated. The "ability to observe in nature" is positively associated with the ratings of wild organisms (Bonferroni-corrected $P = 0.038$), while organisms reared in artificial laboratory or greenhouse environments are positively associated with ratings of seven characteristics: "high fecundity" ($P = 5.44 \times 10^{-4}$), "short generation time" ($P = 0.002$), the ability to "grow in the lab/greenhouse" ($P = 4.33 \times 10^{-8}$) and to "cross in the lab/greenhouse" ($P = 7.03 \times 10^{-6}$), and the availability of "genetic tools" ($P = 1.05 \times 10^{-4}$), "genomic resources" ($P = 0.008$), and a "stock center" ($P = 0.006$). These factors, which combine intrinsic characteristics like fecundity and extrinsic factors like whether a system has enough active researchers to warrant the development of a stock center, can be used to describe the extent to which a study system is broadly thought of as a model organism. These results reflect the historical trajectory of speciation genetics work, which has been conducted mainly in model organisms using a laboratory or greenhouse and in natural systems with few available experimental resources.

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lying them: they are "phenotype-aware" (Barrett and Hoekstra 2011). Admixture mapping uses recombination in natural hybrid zones to map the genetic basis of phenotypic traits that contribute to reproductive isolation. By leveraging multiple generations of natural hybridization, admixture mapping avoids the need to generate crosses, and the elevated recombination in hybrid zones can achieve finer resolution than QTL analyses. Admixture mapping is also conducted in the ecological setting where speciation is occurring (i.e., where the full breadth of selection pressures exists) and can focus on traits that cannot be expressed in the laboratory or greenhouse (Hewitt 1988; Rieseberg and Buerkle 2002; Buerkle and Lexer 2008). For example, this approach was used to map seasonal migration in a hybrid zone between two subspecies of songbirds (Delmore et al. 2016). These subspecies take different routes during migration; their hybrids take intermediate and ecologically inferior routes (Delmore and Irwin 2014; Justen et al. 2021). Hybrids were fitted with archival tags and tracked over the entire annual cycle. Variation in their migratory routes was mapped to a single region on one chromosome. Single-nucleotide polymorphisms (SNPs) in this region were additively inherited and occurred in genes with functions relevant for migration (e.g., *CLOCK*, one of the main components of the circadian clock that allows organisms to respond to changes in photoperiod that initiate migration). Estimates of genomic differentiation between pure forms were also elevated in this region, connecting this behavioral trait with divergent selection (Delmore et al. 2016).

"Bottom-Up" Phenotype-Naive Approaches

Top-down approaches remain restricted to organisms that can be crossed or for which a natural hybrid zone with extensive recombination exists (Buerkle and Lexer 2008). Top-down approaches are also limited to easily observed traits already believed to be involved in speciation. This drawback could produce ascertainment bias in the mechanisms of reproductive isolation that are reported to occur in various taxa. Accordingly, a complementary set of analyses, termed "bottom-up" approaches, has developed. Bottom-up approaches do not require knowledge of phenotypic traits and generally involve using NGS to scan the genome for molecular signatures of reproductive isolation, often called "barrier" loci (Barrett and Hoekstra

2011; Ravinet et al. 2017; Westram et al. 2022). Many forms of bottom-up approaches have expanded the representation of non-model systems in speciation genetics. Indeed, whole-genome sequencing was the most frequently reported genetic tool in our survey of study systems (Supplemental Fig. S7).

For example, genomic clines are used to quantify patterns of introgression across the genome. Loci with restricted patterns of introgression are candidates for barrier loci (Gompert and Buerkle 2011, 2013; e.g., in butterflies [Gompert et al. 2012], songbirds [Parchman et al. 2013], and *Populus* trees [Chhatre et al. 2018]). Ancestry disequilibrium can identify barrier loci, because loci exhibiting nonrandom associations of ancestry in hybrid populations likely underlie incompatibilities and generate reproductive isolation (Schumer and Brandvain 2016; e.g., in swordtail fishes [Schumer et al. 2014], *Drosophila* [Pool 2015], conifers [Menon et al. 2021], stickleback [Thompson et al. 2022], and baboons [Vilgalys et al. 2022]). Patterns of genomic differentiation can also be used to identify barrier loci. This work often uses closely related but divergent populations and assumes that loci showing elevated differentiation are experiencing divergent selection and are involved in maintaining reproductive isolation (Nosil and Feder 2012; Ravinet et al. 2017; e.g., in butterflies [Nadeau et al. 2013], sunflowers [Renaut et al. 2013], *Drosophila* [Kang et al. 2016], and songbirds [Han et al. 2017]). As highlighted by the species cited here, the availability and quality of genomic tools has diversified the taxa in which speciation research is being conducted.

Bottom-up approaches have permitted novel insight into the number and location of barrier loci throughout the genome. Perhaps most importantly, they underscore that speciation can proceed through a few focal changes and does not always require divergence across the entire genome. As speciation proceeds, the number of barrier loci increases, especially in areas of reduced recombination (Wu 2001; Nadeau et al. 2013; Marques et al. 2016; Burri 2017; Delmore et al. 2018; Stankowski et al. 2019). This pattern may have been evident in early genetic models, but its predominance in natural, non-model systems was only revealed with the availability of NGS.

The value of using genome-scale approaches is exemplified by work implicating a role for structural chromosomal variation in speciation (Kirkpatrick and Barton 2006; Wellenreuther and Bernatchez 2018; Faria et al. 2019; and see Berdan et al. 2023 and Lucek et al. 2023). Structural variants, which can create hybrid incompatibilities, are often hinted at by short-read data but need validation with sequencing platforms that generate longer reads, because structural variants are often longer than short reads and have repeat-rich regions making them difficult to map (Bendixsen et al. 2021). Chromosomal inversions have been shown to underlie ecologically important traits, which can facilitate adaptive divergence and potentially speciation by reducing recombination and shielding genomic regions from introgression (e.g., in monkey flowers [Lowry and Willis 2010; Coughlan and Willis 2019], birds [Lamichhaney et al. 2016; Weissensteiner et al. 2020], and flies [Fuller et al. 2018]).

Bottom-up approaches do have drawbacks. For example, genomic clines and scans of ancestry disequilibrium require the existence of hybrid zones of a particular age and large numbers of individuals (Schumer and Brandvain 2016). Further, processes other than speciation can generate genomic patterns indicative of barrier loci. For example, reduced recombination rates (e.g., in inversions or near centromeres) can extend the effects of both positive and negative (or purifying) selection (Noor and Bennett 2009; Turner and Hahn 2010; Cruickshank and Hahn 2014; Delmore et al. 2015; Burri 2017). Bottom-up approaches may also be limited to identifying signatures of reproductive isolation caused by simple genetic architectures and genes of large effect. This approach remains a challenge for identifying polygenic signals of reproductive isolation. Indeed, a recent meta-analysis (Thompson et al. 2023) suggests that ecological speciation often operates through selection on many loci with small-effect alleles, leading to gradual phenotypic divergence. Likewise, incompatibilities between species may often have

a polygenic basis. Finally, there are bioinformatic and financial challenges to using bottom-up approaches in organisms with large genomes (as measured in base pairs, chromosome numbers, and/or ploidy). These challenges limit our understanding of speciation in certain taxa, such as some amphibians and plants. Continual improvements in long-read sequencing and genome assembly methods should make large and complex genomes more accessible (e.g., the 32 Gbp axolotl genome [Nowoshilow et al. 2018] and the maize genome, which comprises ~85% transposable elements [Jiao et al. 2017]). Such advances will enable a better understanding of potential roles for structural rearrangements, polyploidy, repetitive sequences, and transposable elements in speciation.

OPPORTUNITIES FOR SPECIATION GENETICS AT THE INTERSECTION OF LABORATORY AND NATURE

As we describe above, an emerging genomics-enabled transformation is evident in the transition from classical to contemporary studies of speciation genetics. Our practitioner survey confirmed that, while work progresses in traditional model organisms, it is now paired with an expansion in the taxonomic representation of systems within contemporary empirical studies of speciation genetics (Box 1). These findings, and our own experiences as speciation geneticists, also clearly reflect ongoing differences in both the perception and practical reality of "lab" or "greenhouse" versus "wild" systems (Fig. 1; Box 1). Practically, the reciprocal development of laboratory and wild systems for speciation genetics is still maturing and continues to face significant hurdles. Many organisms reared in artificial laboratory or greenhouse environments are likely to perform poorly under ecologically realistic conditions, and the release of manipulated laboratory systems into natural contexts presents logistical, legal, and ethical challenges. Conversely, detailed functional assessment, including newer technologies like CRISPR-Cas9 transformation, remains challenging or impossible in some non-model "wild" systems. Few systems, it seems, are currently able to "do it all."

Given this reality, below we highlight some areas where we envisage the future movement of approaches and knowledge from wild to artificial contexts and vice versa. These efforts could redress historical contingencies in taxonomic diversity and enrich previous analyses of the genetics of speciation. To illustrate these opportunities, we use examples from a few systems that have fruitfully begun to bridge this gap. These examples do not exhaustively describe the literature. Instead, they reflect our own experience and expertise in expanding classical artificially reared systems to natural environments, bringing classical field systems into laboratory contexts, or exploring the merging of these systems and approaches in both of these directions.

From Nature to the Laboratory

The continued development and expansion of wild systems into laboratory and greenhouse (or laboratory- or greenhouse-adjacent) systems offer several advances for our understanding of speciation. One of the greatest benefits of this expansion is the opportunity to identify loci responsible for reproductive isolation under ecologically relevant natural contexts. Wild systems are uniquely situated to address classical questions about how often, and via which mechanisms, selection contributes to speciation. Ideally, identifying genetic variants that cause reproductive isolation should be coupled with investigating the evolutionary forces acting on those genes or mutations under natural conditions. This integration of genetic and ecological context will require moving knowledge from natural populations into artificial laboratory or greenhouse environments. Two wildly successful examples of this involve stickleback fishes and monkey flowers.

Stickleback fishes contain diverse sympatric and parapatric species pairs that vary in divergence times and the magnitudes of gene flow. Ecological studies of postglacial species pairs of the threespine stickleback (*Gasterosteus aculeatus*) have shown that divergent adaptation to contrasting environments can drive the evolution of reproductive isolation (Schluter 2000;

McKinnon and Rundle 2002). Because artificial crosses of these species pairs can be easily made, genetic architectures of ecologically relevant morphological traits have been investigated using QTL mapping (Peichel and Marques 2017). In several cases, causative genes have been identified using fine mapping and transgenic experiments (Colosimo et al. 2005; Peichel and Marques 2017). A combination of artificial crossing and semi-natural pond experiments revealed complex genotype–phenotype fitness relationships of hybrids in semi-natural environments (Arnegard et al. 2014). The closely related species *Gasterosteus nipponicus*, which diverged from the threespine stickleback about 680,000 yr ago (Ravinet et al. 2018), shows hybrid male sterility and courtship behavior divergence compared to the threespine stickleback (Kitano et al. 2009). QTL mapping revealed that hybrid sterility and courtship behavior divergence are controlled by sex chromosomes (Kitano et al. 2009). Sticklebacks belonging to the genus *Pungitius* include sympatric and parapatric species pairs that diverged at more ancient times, such as 1.7 million yr ago, but a low level of ongoing gene flow exists in some species pairs (Yamasaki et al. 2020). Because fertilized eggs can be obtained, and laboratory rearing is possible for most species, further detailed molecular studies are possible using genome-editing technologies (Ansai and Kitano 2022; Kitano et al. 2022). These stickleback species pairs thus provide valuable opportunities to link ecological studies in nature and genetic studies in the laboratory.

The monkey flowers have long been studied in ecology and evolution (Wu et al. 2008), and researchers continue to expand the species under investigation and the approaches used. For example, the *Mimulus aurantiacus* species complex comprises seven closely related subspecies that radiated across California over the past million years (Chase et al. 2017). The two best-studied taxa are very early in the speciation process and display extensive phenotypic differences in their flowers, despite the presence of ongoing gene flow and a highly admixed hybrid zone (Sobel and Streisfeld 2015). Field experiments revealed pollinator isolation caused by divergence in floral traits, including a major shift in flower color (Streisfeld and Kohn 2007). Genetic mapping, combined with association studies in the hybrid zone and virus-induced gene silencing, identified allelic variants in the *MaMyb2* gene responsible for this difference in flower color (Streisfeld et al. 2013). The recent development of further genomic resources (Stankowski et al. 2019) facilitated the discovery that introgressive hybridization deep in the evolutionary history of this radiation fueled the repeated origins of red flower color across the complex (Short and Streisfeld 2023). Despite the presence of a large effect locus controlling flower color differences, a genome scan of geographic variation in ancestry revealed numerous barriers to gene flow on all chromosomes. Curiously, QTLs for floral divergence were not associated with these putative barrier loci, indicating that additional forms of reproductive isolation are also necessary to maintain these distinct taxa (Stankowski et al. 2023). Ongoing work in this system is primed to leverage the extensive phenotypic diversity and multiple natural hybridization zones to investigate the intrinsic and extrinsic forces that keep taxa isolated despite historical and ongoing gene flow.

As these two examples suggest, studying speciation genetics in a laboratory or greenhouse context works most successfully in systems with ecologically diverse wild species where one or more genotypes or species with "laboratory-like" features can be developed as functional laboratory models (Kitano et al. 2022). In wild systems that have greater logistical challenges to expanding into laboratory contexts, some of the best opportunities to identify the genetics of speciation in ecologically informed contexts will be through investigation of natural hybrid zones. For example, admixture mapping has been applied successfully in several taxa to identify loci associated with reproductive barriers (e.g., in mice and rabbits; Ďureje et al. 2012; Turner and Harr 2014; Rafati et al. 2018). Researchers could further adopt this approach by leveraging hybrid zones already described in the classical natural history literature (e.g., Stebbins 1950; Mayr 1963; Remington 1968; Grant 1971). Admixture mapping can also be combined with other data sets to refine lists of can-

didate loci, such as focusing on SNPs identified in admixture mapping and comparative analyses of gene expression, chromatin accessibility, and/or methylation status (Bengston et al. 2018; Laine et al. 2022). In systems without current access to functional tools, related species could be used for functional work on such loci. For example, loci identified in natural avian systems could be validated using zebra finches, where methods for genome editing are under active development (Ahmadiantehrani and London 2017; London 2020; Spool et al. 2021). Such approaches could be implemented in any system where developing genomic resources and data are possible, even if direct functional analysis might not be feasible in the foreseeable future.

From the Laboratory to Nature

Complementary to the transition of wild systems into laboratory contexts, a critical goal of speciation genetics is to identify ecological forces relevant to speciation by transferring the knowledge gained in laboratory- and greenhouse-reared genetic model systems into nature. Many model systems are experimentally and functionally flexible but ecologically uninformed. Pairing them with complementary wild systems offers new opportunities to use natural variation to enhance the ecological and evolutionary annotation of genes and mutations that have been mechanistically described in model systems.

An example that shows how genome-sequencing technology has helped blur the distinction between model and wild organisms is the discovery of a natural hybrid lineage of the yeast *Saccharomyces paradoxus* (the sister species of the laboratory model *Saccharomyces cerevisiae*). Collection of wild samples, short- and long-read genome sequencing, extensive phenotyping, and laboratory crosses have all helped to reconstruct the evolutionary history of these yeasts. This work also identified chromosomal rearrangements at least partially responsible for the reproductive isolation of the hybrid species from both parental lineages (Leducq et al. 2016; Eberlein et al. 2019; for review, see Stelkens and Bendixsen 2022). Likewise, investigations in *Drosophila* have elegantly blended classical genetic approaches like crosses with population genomics of natural populations to investigate gene flow patterns and barrier loci (Meiklejohn et al. 2018).

Promising opportunities to apply ecological context to speciation genetics work also exist in the *Caenorhabditis* genus. These worms, including *Caenorhabditis elegans*, have been studied extensively as genetic model organisms, but little has been known about their ecology until relatively recently (e.g., Kiontke and Sudhaus 2006; Schulenburg and Félix 2017). Alleles contributing to reproductive isolation have been identified in artificial (laboratory-based) crosses between genetically diverse populations, such as alleles involved in parental-effect toxin–antidote-style elements in *C. elegans* (Seidel et al. 2011; Ben-David et al. 2017), *Caenorhabditis tropicalis*, and *Caenorhabditis briggsae* (Ben-David et al. 2021). The phylogeographic population structure of *C. briggsae* (Cutter et al. 2006) raised the possibility that adaptation to temperature could drive genetic incompatibilities between populations. Inter-chromosomal linkage disequilibrium is modulated by temperature in within-species *C. briggsae* hybrids (i.e., genotype-by-genotype-by-environment, or G×G×E effects), and investigating the genomic regions that are co-inherited depending on the environment might identify loci that cause reduced fitness (Cazares-Navarro and Ross 2019). These current efforts, which integrate genetic investigation and ecological factors, are identifying alleles that are potentially incompatible in within-species hybrids.

Extensive genomic development of agricultural model species has also generated infrastructure and comparative genomic data that can be repurposed for tests of evolutionary questions in natural contexts. For example, 32 whole genomes of 11 *Solanum* species, which were originally generated for research in the domesticated tomato *Solanum lycopersicum*, were used to evaluate clade-wide patterns of introgression prevalence among wild tomato species (Hamlin et al. 2020). These data showed that introgression was frequently detectable among wild species but was modest in scope (~0.5%–2% of

the genome). Moreover, across multiple species comparisons, introgression was more prevalent between geographically proximate populations and between species that share mating systems; introgression also tended to decrease as genetic divergence increased between species. These results suggested that several biological factors, like reproductive proximity and time since common ancestry, broadly shape the frequency of genetic exchange across the clade. Similar analyses in "model-adjacent" wild systems could capitalize on existing genomic data to clarify these or other (e.g., Moyle and Nakazato 2010; Pease et al. 2016) general patterns of reproductive isolation across diverse organisms and ecological contexts.

These examples show that bringing genetic data and knowledge from traditional model systems into wild contexts provides rich opportunities to assess the ecological conditions or evolutionary forces acting on individual genetic variants and supports the identification of general patterns of divergence and reproductive isolation in closely related wild systems.

Integrating Laboratory and Nature

Given the complementary value of model and wild systems, an ideal approach for the future study of speciation genetics and genomics would be to integrate top-down and bottom-up approaches by applying laboratory-based techniques to natural systems and ecological information from wild taxa to laboratory- and greenhouse-reared systems (Fig. 2). As we have noted, studies that combine the merits of both genetic models and genomic tools from wild species will likely be most impactful in the foreseeable future (Stankowski et al. 2023). Some of these systems were clearly represented in our survey of speciation genetics researchers, including some taxa that were represented by multiple responses, where the research community believed their study system spanned the spectrum from studying wild populations to laboratory-adapted ones (Fig. 1). Regardless of where these (and other) species fit along this continuum, they all provide excellent opportunities to integrate top-down and bottom-up approaches: combining laboratory- and greenhouse-based research, including genetics and genomics, with field experiments and observations that provide an ecological context to the study of speciation.

CONCLUDING REMARKS

Expanding the genomic analyses of reproductive isolation into natural populations, especially in longer-lived and lower-fecundity species, promises to inform us about how genetics and ecology drive speciation. Conversely, the application of functional tools and knowledge from laboratory models to wild systems has enormous potential to provide mechanistic insights into species formation under more ecologically realistic conditions. Although few systems can likely span the full gamut from ecological studies in the wild to molecular genetic studies in the laboratory, insights obtained from natural populations and laboratory organisms are clearly complementary and ultimately necessary for a complete understanding of the genetic basis of speciation. This empirical diversity need not imply that our field is becoming less theoretically driven. Instead, it suggests that we now face an unprecedented opportunity to empirically evaluate longstanding speciation theory across more biological systems and contexts. What remains to be seen is whether new or different patterns emerge from expanding the reach of speciation genomics beyond traditional systems. Regardless of whether this expanded picture spurs new theory and new expectations, it is bound to generate a more inclusive assessment of both the genetic changes and evolutionary forces that characterize the formation and persistence of new species.

METHODS

We surveyed the speciation genetics/genomics research community using an instrument developed by the authors. The Committee for the Protection of Human Subjects at California State University, Fresno approved this research (protocol #1387). One hundred and sixty-four responses were collected. Following quality control, the final data set comprised 131 responses.

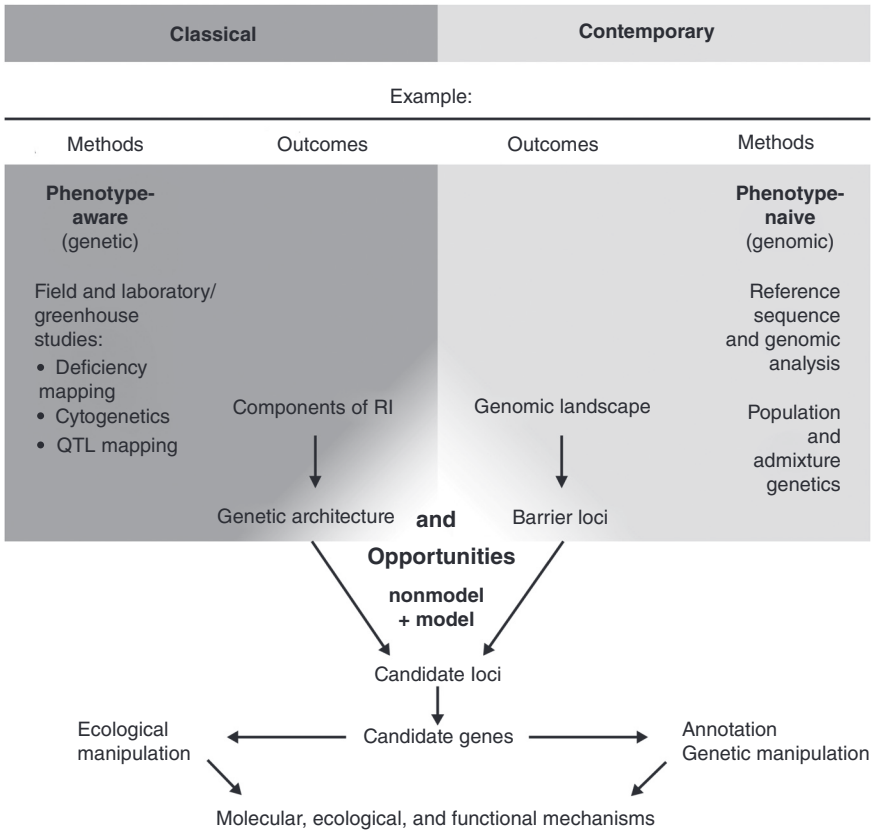

Figure 2. Approaches and goals of speciation genetics studies over time. Two main approaches have been used to investigate the genetic and genomic architectures of reproductive isolation (RI) and identify candidate loci responsible for reproductive isolation. Classical genetic methods were traditionally used in phenotype-aware studies, and genomics has facilitated phenotype-naive approaches for more taxa. Future opportunities integrate knowledge gained from both approaches to understand the ecological context of speciation genetics in traditional laboratory organisms and to identify the genetic basis of reproductive isolation in wild or peri-model species.

The instrument and additional methodological details are presented in Supplemental File S1. The de-identified response data (all demographic data removed) are available in Supplemental File S2.

AUTHOR CONTRIBUTIONS

Writing: K.D., H.J., K.M.K., J.K., L.C.M., R.S., M.A.S., Y.Y.Y., J.R.; Methodology: K.D., H.J., K.M.K., J.K., L.C.M., R.S., M.A.S., Y.Y.Y., J.R.; Investigation, Data curation, and Data visualization: J.R.; Formal analysis: Y.Y.Y., J.K.

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