

NEWS AND VIEWS

MEETING REVIEW

Genomic studies on the nature of species: adaptation and speciation in *Mimulus*

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Evolutionary biology is in an exciting era, in which powerful genomic tools make the answers accessible to long-standing questions about variation, adaptation and speciation. The availability of a suite of genomic resources, a shared knowledge base and a long history of study have made the phenotypically diverse plant genus *Mimulus* an important system for understanding ecological and evolutionary processes. An international *Mimulus* Research Meeting was held at Duke University in June 2014 to discuss developments in ecological and evolutionary genetic studies in *Mimulus*. Here, we report major recent discoveries presented at the meeting that use genomic approaches to advance our understanding of three major themes: the parallel genetic basis of adaptation; the ecological genomics of speciation; and the evolutionary significance of structural genetic variation. We also suggest future research directions for studies of *Mimulus* and highlight challenges faced when developing new ecological and evolutionary model systems.

Keywords: adaptation, genomics, *Mimulus*, parallel evolution, speciation, structural variation

Received 31 January 2015; revised 25 March 2015; accepted 27 March 2015

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Introduction

A central goal in evolutionary biology is to link adaptive phenotypes in natural populations with their underlying genetic mechanisms (Barrett & Hoekstra 2011). Research in ecological and evolutionary genetics is taking advantage of novel genomic technologies to investigate a series of questions that were, until recently, beyond reach (Stapley *et al.* 2010; Andrews *et al.* 2013; Seehausen *et al.* 2014). Some of these questions include: What is the genetic and genomic architecture of adaptive change? What components of parallel evolution occur through a shared genetic basis? How much of the genome is exposed to selection? How does genome structure evolve, and how does this affect adaptation and speciation? Does standing genetic variation act as a source of alleles for the evolution of reproductive isolation? While progress is occurring, significant advances will be most likely when the shared knowledge and resources, available for classic model systems, are developed in groups that have a rich natural history and ecology.

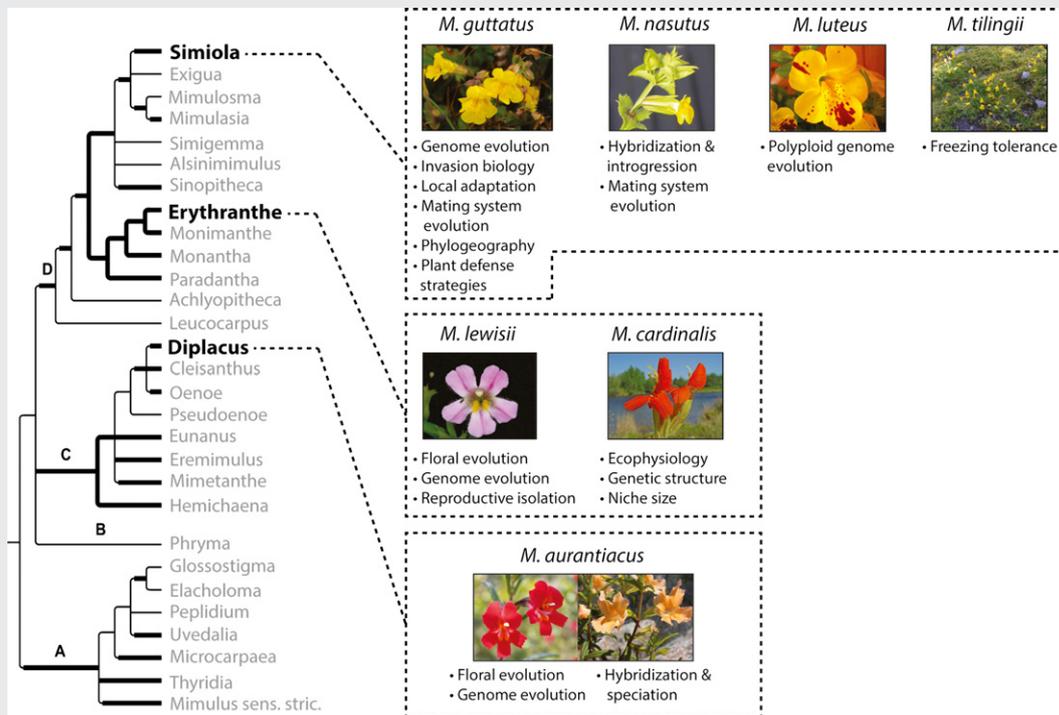
The success of ecological and evolutionary genomic research in any organismal system depends on having a strong community of diverse researchers that builds intellectual and material resources (Feder & Mitchell-Olds 2003). Those working in model systems, including *Drosophila* and *Arabidopsis*, have long realized the need for community resources, including germplasm centres, mutant lines and molecular databases, all of which require collaboration across multiple research laboratories at different institutions. In contrast, ecologists and evolutionary biologists traditionally have been more independent, focusing instead on conceptually overlapping ideas across a broader set of taxa. The recent, widespread availability of genomic technology has accelerated the development of collaborative research communities working in new model systems that address fundamental questions in ecology and evolution (Colbourne *et al.* 2011; Joron *et al.* 2011; Jones *et al.* 2012). In this review, we focus on recent advances made in understanding adaptation and speciation through collaborative and complementary research conducted by the community of scientists focused on the ecological and evolutionary model plant genus, *Mimulus*.

Monkey flowers (*Mimulus*, Phrymaceae) have a long and venerable history of providing key insight into fundamental evolutionary processes (Box 1). While research on *Mimulus* began with Darwin (1876), modern studies of *Mimulus* were propelled by the classic works of Clausen, Keck and Hiesey (Clausen *et al.* 1940; Clausen & Hiesey 1958). Robert Vickery, a graduate student of Jens Clausen, launched the first investigations of local adaptation in *Mimulus* (Vickery 1952) by studying populations of *M. guttatus* along a transect from coastal to inland California

Box 1: Natural history and past research in *Mimulus*

Mimulus sensu lato is a genus of at least 170 species, most of which are herbaceous annuals or perennials associated with moist or wet soils. The genus is globally distributed with a lineage of approximately 7 species centred in Australia and a larger American-Asian radiation of 165 species (Barker *et al.* 2012; Fig. 1). The group is best known for its ecological and morphological diversity that includes, among other things, extensive variation in life history, mating system, pollination and edaphic specialization (Wu *et al.* 2008). While variation among closely related taxa makes them ideal for evolutionary studies, molecular phylogenies in the genus are limited to only a few genes (Beardsley *et al.* 2004), preventing resolution of several important nodes. However, progress in systematic research includes a taxonomic framework for the whole family (Barker *et al.* 2012) that will be further improved by phylogenomic approaches that take advantage of ongoing genome resequencing of representatives across the group (J. Willis, personal communication).

Evolutionary research in *Mimulus* has focused primarily on three taxonomic groups in North America, highlighted in Fig. 1. Section *Simiola*, the clade containing *M. guttatus*, has been well studied in terms of mating system evolution, speciation and reproduction isolation, as well as adaptation (e.g. Ritland & Ritland 1989; Martin & Willis 2007; Lowry & Willis 2010). Species in the section show a wide range of adaptive variation, including tolerance to copper-rich soils in *M. cupriphilus*, serpentine tolerance in *M. nudatus*, the evolution of selfing in *M. nasutus* and adaptation to high altitude in *M. tilinigi* and *M. laciniatus*. Section *Erythranthe*, containing *M. lewisii* and *M. cardinalis*, is best known for research on the genetic basis of reproductive isolation, pollinator preference and the evolution of geographic range limits (Schemske & Bradshaw 1999; Bradshaw & Schemske 2003; Angert 2006, 2009). Finally, Section *Diplacus* is a radiation of closely related woody, perennial subshrubs across California, with *M. aurantiacus* as an example of divergent ecotypes maintained by pollinator reproductive isolation (Grant 1981; Streisfeld *et al.* 2013).



Box 1 Fig. 1 Evolutionary relationships and research areas for well-studied *Mimulus* taxa. Phylogeny of major sections in *Mimulus* and related genera in the Phrymaceae, highlighting the clades and taxa where current research focuses on adaptation and speciation. The major research aims addressed in each species are listed below photographs. Phylogeny adapted from Barker *et al.* (2012), which depicts the major clades from the study of Beardsley *et al.* (2004). Branches in bold have bootstrap support values >80%. Clade labels are as follows: (A) Old World lineage, (B–D) American-Asian lineage. Picture credits: Lila Fishman, John Game, George Hartwell, Gary Monroe, Šarūnas Šimkus, Matthew Streisfeld and Alex Twyford.

(Clausen *et al.* 1940; Clausen & Hiesey 1958). Vickery continued a prolific career working on the evolution of postzygotic reproductive isolation in *Mimulus* (e.g. Vickery 1964, 1978). William Hiesey first investigated the evolution of pollinator divergence between *M. cardinalis* and *M. lewisii* (Hiesey *et al.* 1971). Douglas Schemske and Toby Bradshaw continued this line of research with pioneering quantitative trait locus (QTL) mapping studies in which they identified major loci underlying the evolutionary transition between bee- and hummingbird-pollinated flowers (Bradshaw *et al.* 1995; Bradshaw *et al.* 1998). In a series of now classic works in ecological genetics, they then linked the pollinator shift to the process of speciation (Schemske & Bradshaw 1999; Bradshaw & Schemske 2003). In a similar vein, Verne Grant incorporated knowledge of floral divergence between hybridizing taxa in the *M. aurantiacus* species complex to develop his concept of floral isolation (Grant 1981, 1993). Major contributions were made to our understanding of mating system evolution and inbreeding depression through research on *Mimulus* (e.g. Ritland 1989; Willis 1993; Fenster & Ritland 1994; Dudash & Carr 1998; Fishman *et al.* 2002). In addition, Mark Macnair furthered the field's knowledge of the genetic architecture of adaptation to extreme edaphic environments, including copper mines and serpentine soils (MacNair 1983; Macnair & Gardner 1998). The wealth of knowledge amassed by these and other researchers, as well as the potential for future advances, resulted in the 2005 decision by the Joint Genome Institute (DOE) to make *Mimulus* one of its pioneer plant species for whole-genome sequencing.

In 2008, Wu *et al.* described five features of *Mimulus* that positioned it as an emerging model system for studies of ecological and evolutionary functional genomics: (i) its widespread ecological diversity, (ii) the evolutionary relevance of this diversity, (iii) possessing the attributes of a classic genetic model (e.g. short generation time, high fecundity, small size), (iv) the existence of a broad research infrastructure and (v) availability of genomic resources (also see Feder & Mitchell-Olds 2003). Importantly, many of these attributes applied to several species in the genus (see Box 1), making the group as a whole an excellent system for studying evolution. However, at the time, genomic resources were primarily available for only one species (*M. guttatus*), and even these were poorly developed.

Over the decade since genome sequencing began in *Mimulus*, the development and use of genomic tools across the entire genus has increased remarkably, which has provided novel insight into the processes of adaptation and speciation. Here, we highlight research developments and emerging themes from the recent biennial *Mimulus* Research Meeting held at Duke University, Durham, NC (19–20 June 2014), focusing on areas that are particularly fruitful using a genomic approach: the parallel genetic and genomic basis of adaptation, the ecological genomics of speciation and the evolutionary consequences of structural genetic variation. In addition to covering these key topics, we describe ongoing challenges in the continued improvement of *Mimulus* as a model and conclude with recent

developments that make future studies uniquely positioned to connect genotype, phenotype and fitness in nature. By integrating across studies in *Mimulus*, we illustrate its promise as a model system that provides the necessary breadth and depth to illuminate general patterns of evolution in the genomic era.

Parallel adaptation and the repeated outcomes of evolutionary change

The repeated, independent evolution of similar characters—parallel evolution—often reflects adaptation caused by similar selection pressures. Multiple origins of the same trait offer natural replication and provide an opportunity to explore the types of constraints that dictate the course of phenotypic evolution (Kopp 2009). An important question that emerges from these studies is whether the genetic changes contributing to repeated phenotypic evolution are predictable (Conte *et al.* 2012) and whether natural selection preferentially targets specific genes—or certain types of mutations—during adaptation. In *Mimulus*, there are numerous examples of parallel adaptation, such as the repeated shifts in flower colour (Cooley *et al.* 2011; Streisfeld *et al.* 2013; Wu *et al.* 2013), pollination syndrome (Beardsley *et al.* 2003), tolerance to edaphic environments (Macnair & Gardner 1998) and mating system (Ritland 1989; Fenster & Ritland 1994; Fishman *et al.* 2002, 2014a). Recent studies in *Mimulus* are leveraging genetic and genomic technologies to provide insight into whether the adaptive process is predictable (Soria-Carrasco *et al.* 2014).

Adaptive transitions in flower colour are common in nature due to their prominent role in the attraction of animal pollinators (Grant 1993; Fenster *et al.* 2004). Flower colour changes caused by altered levels of anthocyanin pigmentation are well studied and frequently involve mutations in the *R2R3-MYB* family of transcription factors that are expressed in a tissue-specific manner with minimal pleiotropy (Rausher *et al.* 2008; Streisfeld & Rausher 2011). While most transitions result in loss of pigmentation, at least four examples between closely related *Mimulus* taxa involve gains of pigmentation (reviewed in Sobel & Streisfeld 2013). Surprisingly, three of these examples also involve mutations in *R2R3-MYB* transcription factors (Cooley *et al.* 2011; Streisfeld *et al.* 2013), revealing the remarkable specialization of these genes for flower colour evolution. However, elevated anthocyanin levels in *M. cardinalis* are due to a mutation in a different class of regulatory protein (*R3-MYB*) that blocks repression of the anthocyanin pathway (Yuan *et al.* 2013b). Thus, these results reveal both the predictable nature of flower colour evolution and how a mechanistic approach can provide insight into the adaptive process. At the meeting, Yaowu Yuan (University of Connecticut) argued that differences in carotenoid pigmentation also have evolved multiple times in *Mimulus*, opening up new possibilities for the study of parallel evolution and predictability. Carotenoid biosynthesis and its regulation are poorly understood in all plants, and so *Mimulus* may be an ideal system for understanding the molecular genet-

ics of this important class of pigments. Overall, the topic of linking parallel phenotypic changes with the corresponding genetic changes represents a fruitful area of future research that will allow tests for repeatability across different temporal and spatial scales.

The flowering time pathway is another well-characterized developmental pathway in plants (Putterill *et al.* 2004; Andrés & Coupland 2012) that has experienced repeated selection. By flowering in response to predictable changes in day length, plants can avoid unfavourable environmental conditions and synchronize their flowering. Several studies have shown that strong selection to avoid summer drought has shaped seasonal phenology in annual populations of *M. guttatus* (Hall *et al.* 2006; Lowry *et al.* 2008; Friedman & Willis 2013; Fishman *et al.* 2014a). In California and Oregon, where seasonal drought is common, the start of the growing season for *M. guttatus* often varies with altitude. For example, flowering in spring (i.e. in shorter day lengths) may be advantageous at low elevations because soils dry out rapidly in the summer. However, flowering in summer (i.e. under long day lengths) may be advantageous at high elevations to avoid late season snowfalls (Kooyers *et al.* 2015). Ben Blackman (University of Virginia) presented new results on the genetic basis of clinal differentiation for critical photoperiod across altitudinal gradients in the Sierra Nevada and Cascade mountains. Using high-throughput sequencing approaches for QTL mapping across multiple, parallel altitudinal transects, Blackman demonstrated that few loci for critical photoperiod are shared between transects, and none are consistently found across the range studied, suggesting that parallel phenotypic divergence has evolved through different genetic changes. Further experimental studies in this system will be required to determine whether the few, shared QTLs are caused by alleles of the same or linked genes and whether the diverse evolutionary outcomes reflect parallel adaptation (Ralph & Coop 2010).

In addition to research focusing on well-characterized pathways, genomic studies provide an excellent opportunity to examine the parallel adaptation of novel traits. Heavy metal-contaminated soils present strong selective pressures on local plant communities (Gregory & Bradshaw 1965). Populations of *M. guttatus* have repeatedly colonized copper-contaminated soils at mine sites throughout California (Allen & Sheppard 1971), and genetic mapping revealed that a single locus of large effect underlies tolerance to elevated copper levels (MacNair 1983; Wright *et al.* 2013). To identify the gene underlying this locus, and additional loci that may contribute to fitness variation in the copper-contaminated soils, Kevin Wright (Harvard University), in collaboration with Uffe Hellsten and Dan Rokhsar (Joint Genome Institute), conducted whole-genome sequencing from pools of plants from mine and off-mine populations. Wright presented comparisons of the difference in allele frequencies segregating between habitats, which allowed them to identify candidate genes that may underlie copper mine adaptation. This work demonstrates how detecting the signature of heterogeneous genomic

divergence in natural populations can reveal genes that may be important for adaptation to stressful soil environments (Roesti *et al.* 2014). By extending this research to other copper-tolerant populations of *M. guttatus*, researchers can test whether parallel adaptation involves the same loci, either through recurrent mutation or through recruitment from standing genetic variation. More generally, by comparing the loci underlying adaptation to other extreme soil conditions, such as serpentine soils or soils adjacent to thermal hot springs (Lekberg *et al.* 2012), it will be possible to test whether there are common targets of selection in response to diverse edaphic pressures. These studies may be particularly insightful in *Mimulus*, as many species have genetically diverse populations that harbour substantial intrapopulation variation for ecologically relevant traits (e.g. Mojica *et al.* 2012).

Ecological genomics of speciation

Considerable progress has been made in understanding the role that ecological divergence plays in the origins of new species (Schluter 2001; Coyne & Orr 2004; Nosil 2012; Butlin *et al.* 2014). It is now clear that reproductive barriers can evolve as a by-product of ecologically based divergent selection, even in the face of gene flow (Feder *et al.* 2005; Nosil *et al.* 2009; though see Cruickshank & Hahn 2014). The continuity of the speciation process (Coyne & Orr 2004; Hendry *et al.* 2009) means that incompletely isolated taxa are pivotal in identifying the barriers involved in the initial stages of divergence. Studies of speciation in *Mimulus* provide textbook examples of the evolutionary ecology and genetics of reproductive isolation at different stages along this continuum (Fishman & Willis 2001; Bradshaw & Schemske 2003; Ramsey *et al.* 2003; Martin & Willis 2007). Ongoing studies in *Mimulus* are using genomic information to identify the regions of the genome that contribute to reproductive isolation, particularly at early stages.

Mimulus includes numerous ecotypes and species that co-occur and hybridize to various extents. For example, the largely outcrossing species *M. guttatus* and the closely related selfing *M. nasutus* are mostly reproductively isolated due to differences in flowering time, mating system, conspecific pollen precedence and hybrid incompatibilities (e.g. Kiang & Hamrick 1978; Martin & Willis 2007; Fishman *et al.* 2014a; Sweigart & Flagel 2015). Nevertheless, the species continue to produce hybrids in nature, providing an excellent opportunity to examine their speciation history and the extent and consequences of introgression between them. Brandvain *et al.* (2014) used population genomic sequencing to reveal that the more geographically restricted and selfing *M. nasutus* budded off from the geographically more widespread, outcrossing *M. guttatus*. This supports recent evidence from comparative phylogenetics of range sizes that this may be a general pattern of speciation among *Mimulus* taxa (Grossenbacher *et al.* 2014). In addition, hybridization results in introgression between species, but patterns of divergence suggest that selection against hybrids eliminates long chromosomal blocks of

M. nasutus ancestry in *M. guttatus* (Brandvain *et al.* 2014). Amanda Kenney and Andrea Sweigart (University of Georgia) presented genotyping-by-sequencing (GBS) data from a sympatric site with ongoing hybridization, which allowed them to trace the ancestry of individual linkage blocks. This approach may enable identification of loci that are preferentially introgressed and to determine the consequences of this introgression for speciation.

To investigate an earlier stage of divergence, Matthew Streisfeld (University of Oregon) is studying two ecotypes within the widespread *M. aurantiacus* species complex. In a classically described example of incipient speciation (Grant 1981), red-flowered and yellow-flowered ecotypes of *M. aurantiacus* occur parapatrically in southern California, but hybridize in a narrow region of contact (Streisfeld & Kohn 2005). The ecotypes are partially isolated due to premating barriers including ecogeographic isolation and pollinator preference, but postmating barriers are largely nonexistent (Grant 1993; Streisfeld & Kohn 2007; Sobel & Streisfeld 2015). Despite ongoing gene flow between the ecotypes, several floral and ecophysiological traits vary clinally across the hybrid zone, and divergent selection is sufficient to keep the ecotypes distinct (Stankowski *et al.* in review). Streisfeld presented results from additional RAD-sequencing projects that reveal very few differentiated genomic regions, suggesting that selection on a few key loci underlies divergence and the evolution of premating isolation. These data reflect how studying speciation at its earliest stages can reveal the ecological and genomic changes that contribute to reproductive isolation.

Structural genomic changes and their association with adaptation

A major finding from genome sequencing studies is that fixed genomic differences between related species are not restricted to single nucleotide polymorphisms (SNPs), but also include major structural variants such as indels, translocations and inversions (Noor *et al.* 2007; Alkan *et al.* 2011; Ventura *et al.* 2011). Moreover, changes in chromosome number and structure are often important contributors to adaptation and speciation (Hoffmann & Rieseberg 2008; Kirkpatrick 2010). Recent studies in *Mimulus* are revealing extensive structural genomic variation at multiple scales. For example, numerous small inversions have been found to segregate within a population of *M. guttatus* (Flagel *et al.* 2014); large translocations differ between *Mimulus* species and cause pollen sterility (Stathos & Fishman 2014); and chromosomal fission accounts for a near doubling of chromosome number at the base of the clade containing the yellow monkey flowers (Fishman *et al.* 2014b). The origins and maintenance of such structural variation is intriguing, as individuals heterozygous for structural variants often suffer reduced fitness (Stathos & Fishman 2014; Hollister 2015).

One possible impetus for the spread of chromosomal variants, as well as their maintenance as polymorphisms, is selfish evolution via female meiotic drive. Centromeres, which mediate faithful chromosomal segregation as the point of

spindle attachment, can gain a transmission advantage by preferentially moving to the egg pole in asymmetric female meiosis (Henikoff & Malik 2002). Consequently, they can act as selfish genomic regions, causing meiotic drive that can generate hybrid incompatibilities (Orr 2005), maintain deleterious variation (Fishman & Saunders 2008) or promote chromosomal structural evolution. *Mimulus*, which has a known centromeric driver (Fishman & Willis 2005), provides excellent opportunities to investigate patterns and processes of centromere evolution. For example, Findley Finseth presented her results with Lila Fishman (University of Montana) on putative centromeric repeat variation across *Mimulus*, using whole-genome sequence data from a dozen species. Changes in the dominant repeat correspond with changes in chromosome structure (e.g. fission and fusion events) and also with an unusual duplication of the centromere-defining histone CenH3. This talk highlighted the diversity of centromere structure across *Mimulus* and the potential of the system for broad-scale investigation of genome evolution as well as mechanistic studies of individual genomic regions.

Whole-genome duplication (polyploidy) is widespread in plants and has implications for adaptation and speciation. Violeta Simon-Porcar and Pauline Pantoja presented work with Mario Vallejo-Marín (University of Stirling), on *M. peregrinus*, a recently formed allopolyploid species in the British Isles derived from hybridization between introduced populations of *M. guttatus* and *M. luteus* (Vallejo-Marín 2012; Vallejo-Marín & Lye 2013). Joshua Puzey (College of William and Mary) described ongoing work focused on understanding the causes of homoeolog expression biases in this neo-allopolyploid species. As part of this project, Puzey and Arielle Cooley (Whitman College) created a high-quality de novo genome assembly of *M. luteus* based on deep paired-end and mate-pair sequencing. Interestingly, an American allopolyploid, *M. sookensis*, shares a parent progenitor (*M. guttatus*) with allopolyploid *M. peregrinus*, opening the door for unique comparative studies of naturally evolved allopolyploids (Modliszewski & Willis 2012). While polyploidy may be a common feature in *Mimulus* and in plants generally, a history of whole-genome duplications is not the only explanation for the extensive chromosome number variation across *Mimulus* (Vickery 1978). Instead, recent genetic mapping studies suggest that the increase from a base chromosome number of $n = 8$ to $n = 14$ in *M. guttatus* and its close relatives is the product of a burst of chromosomal fission and fusion events (Fishman *et al.* 2014b). This finding challenges the widely held assumption that large increases in chromosome number are always due to polyploidy, and reveals the dynamic processes of genome restructuring that occur in the genus.

These emerging results highlight the lability of genome structure in *Mimulus*. However, it is still unresolved how much of this structural variation is adaptive. Alex Twyford (University of Edinburgh) and Jannice Friedman (Syracuse University) used GBS to study patterns of genetic diversity within and outside a large chromosomal inversion in populations of *M. guttatus*. Although genome-wide patterns

of genetic structure reflect geography, they found a contrasting pattern within the vicinity of a known chromosomal inversion polymorphism, where genetic structure is perfectly associated with life history divergence (annual vs. perennial ecotype) (Twyford & Friedman 2015). Previous work showed the inversion is involved in local adaptation (Lowry & Willis 2010); thus, this result raises the prospect that the inversion has spread due to selection following the capture of locally adapted alleles at multiple loci, as predicted by theory (Kirkpatrick & Barton 2006). An important future goal of this research is to elucidate the molecular mechanism that gives rise to the fitness of chromosomal rearrangements. For example, in sticklebacks, many structural genomic changes disrupt gene function (Feulner *et al.* 2013), whereas in other cases, inversions capture locally adapted alleles and protect them from recombination (Joron *et al.* 2011).

Ongoing development of *Mimulus* as a model evolutionary genomic system

Studies in *Mimulus* have played an important role in advancing our understanding of the genetic basis of adaptation, and it is regarded as a model system for studying adaptive change in plants. Yet many challenges exist for the continued development of *Mimulus* as a model genus. These include logistical difficulties of centralizing resources and making them available to other research groups, but also technical challenges associated with studying the genetic basis of adaptation in phenotypically diverse organisms. These challenges are common to many other systems (Kocher 2004; Bernasconi *et al.* 2009), and we highlight several important developments that will allow for the continued growth of *Mimulus* as a model system.

A major landmark for developing an evolutionary model system is the creation of a high-quality reference genome (Ekblom & Galindo 2011). Although the draft genome assembly for *M. guttatus* (www.phytozome.net; Hellsten *et al.* 2013) includes 14 large linkage groups reflecting the 14 chromosomes, there are numerous assembly errors. Improvements are being made by reassembling the scaffolds with linkage data generated from hybrid mapping populations (Holeski *et al.* 2014), and ongoing projects using whole-genome mapping (formerly optical mapping, Aston *et al.* 1999; Chamala *et al.* 2013). An improved reference genome will be particularly useful for population genomic analyses, as well as for future comparative studies of genome structure and synteny across the genus. In addition to the *M. guttatus* reference genome, short-read draft genome assemblies exist for *M. aurantiacus*, *M. cardinalis*, *M. lewisii* and *M. luteus* and low-coverage sequences for several additional species. There are also a growing number of species with multiple individuals sequenced at high depth, and Josh Puzey and John Willis (Duke University) discussed their highly multiplexed genome resequencing of *M. guttatus* to investigate broad-scale patterns of population structure and signals of localized selection (e.g. Puzey & Vallejo-Marín 2014). This population-level sequencing

approach is similar to the studies of model species such as *Arabidopsis thaliana* (Cao *et al.* 2011).

In addition to the generation of genomic data, an important stage for developing evolutionary model systems is comprehensively linking ecologically relevant phenotypes to their underlying genotypes (Benfey & Mitchell-Olds 2008). This remains a challenge for many ecologically important traits, especially those that are difficult to score reliably or show intra-individual variation (e.g. production of secondary defence compounds, Holeski *et al.* 2014; trichrome density, Holeski *et al.* 2010). The development of high-throughput phenotypic screens is particularly valuable, as they enable reliable scoring of complex phenotypes in large populations. Kathleen Greenham (Dartmouth College) gave one example of a high-throughput phenotypic screen using time-lapse imaging to track leaf movement for investigating variation in circadian period (Engelmann *et al.* 1992). Using this approach, she discovered strong geographically and ecologically relevant variation in circadian period among populations of *M. guttatus*. Such high-throughput phenotyping enables the screening of very large segregating mapping populations, which can increase the power and resolution of QTL mapping as well as other analyses.

After identifying candidate loci, functional genetic analyses are required to confirm gene function, either through RNAi silencing, CRISPR/Cas-9 genome editing, or the generation of knockout mutants or overexpressed transgenic lines (Gase & Baldwin 2012). Yaowu Yuan (University of Connecticut) discussed how his work in *M. lewisii* with Toby Bradshaw (University of Washington) uses *in planta* stable transformation protocols (Yuan *et al.* 2013b) and mutagenesis experiments (Yuan *et al.* 2013a) to confirm the function of flower colour and floral scent genes (Byers *et al.* 2014). Protocols for stable transformation and virus-induced gene silencing (VIGS) are also available for *M. aurantiacus* (Streisfeld *et al.* 2013; Susič *et al.* 2014) and *M. guttatus* (Preston *et al.* 2014). Overall, these approaches add to the available molecular toolkit for evolutionary studies in the genus and represent important progress in understanding the functional genetic basis of complex phenotypes in *Mimulus*.

Conclusions

The value of developing model systems comes in two forms: first, shared resources, which maximize the impact of investment; and second (and most importantly), shared knowledge, which creates opportunities for biological insight greater than the sum of individual research programmes. The community of researchers studying diverse questions across multiple *Mimulus* species has grown rapidly, with it being the second most featured organism, based on presentation titles, at the 2014 Evolution Meeting (see discussion at <https://agreattree.wordpress.com/2014/06/24/evolution-2014>). This focus facilitates both the depth necessary to understand mechanism and the breadth necessary to illuminate general patterns. In the 10 years since genome sequencing began in *M. guttatus*, we have seen remarkable advances in technology that allow unprecedented

insight into classic questions in ecological and evolutionary genetics. One of the most promising outcomes of these and future studies is the potential to integrate detailed functional genomic analyses with ecological data to reveal the direct fitness effects of individual alleles in natural populations. As these dreams become realized, we are excited to see where the field will be 10 years from now.

Acknowledgements

We thank the organizers of the *Mimulus* Research Meeting: Yaniv Brandvain, Joshua Puzey, Andrea Sweigart, John Willis and Duke University for hosting the meeting. We also thank the participants for the stimulating discussion. The manuscript was improved by comments from Ben Blackman, Lila Fishman, Kathleen Greenham, John Kelly, Joshua Puzey, Andrea Sweigart, Mario Vallejo-Marín, Kevin Wright and Yaowu Yuan.

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All authors contributed to the writing of this manuscript.

doi: 10.1111/mec.13190