Title
Dissecting Trait Variation across Species Barriers

Journal
Trends in Ecology & Evolution, 34(12)

ISSN
0169-5347

Authors
Weiss, Carly V
Brem, Rachel B

Publication Date
2019-12-01

DOI
10.1016/j.tree.2019.07.013

Peer reviewed

Published in final edited form as:

Trends Ecol Evol. 2019 December ; 34(12): 1131–1136. doi:10.1016/j.tree.2019.07.013.

Dissecting trait variation across species barriers

Carly V. Weiss[1,3], **Rachel B. Brem**[*,1,2]

[1]Department of Plant and Microbial Biology, UC Berkeley, Berkeley, CA,

[2]Buck Institute for Research on Aging, Novato, CA

[3]Current address: Department of Biology, Stanford University, Stanford, CA

Abstract

Dissecting the basis of naturally occurring trait variation is one of the central goals of modern genetics. For eukaryotes, classic methods for this purpose rely on screens of recombinants from matings between distinct parents. These tools cannot be used in studies of species that can't mate to form recombinant progeny in the first place. But new approaches are coming online to shuffle the genomes of otherwise incompatible species. With them, geneticists can elucidate how evolution built a new trait—even if it happened millions of years ago, in a lineage that's now reproductively cut off from its closest relatives.

Keywords

Statistical genetics; evolution; interspecific variation

The genetics of evolutionary innovation

Over the four billion years that life has evolved on this planet, organisms have acquired astonishing phenotypes. Some capture our attention by their sheer beauty: imagine a lion's mane or a butterfly's wing. Other characters, like silk production in spiders or longevity in naked mole rats, get us excited by their relevance for industry and biomedicine. To figure out how evolutionary innovation happens, and bring its benefits to bear in translational applications, we need to track down the alleles that nature used to evolve a given trait, long ago in history. In this review, we first cover the classic approaches to this problem. We then highlight a family of new techniques that open a heretofore intractable research area—the phenotypic differences between isolated species.

Local adaptation and trait differences between species

If we ask a scientist on the street what experiment to do to dissect the genetics of trait variation in the wild, the answer will likely be, "A genome-wide association study" (GWAS;

[*]Correspondence: rbrem@buckinstitute.org.

see Glossary). In GWAS, we screen for co-inheritance between genotype and phenotype across outbred individuals, whose ancestors exchanged genetic material for many generations before the present. This is indeed a powerful and unbiased approach, as is the related statistical-genetic technique called linkage mapping, which screens recent recombinant progeny from closely related individuals in families.

To use these tools in the study of evolutionary innovation, ecologists classically seek out a group of individuals that flourish in a unique niche but can still mate with members of the same species elsewhere [1, 2]. The goal then is to find the alleles underlying a given trait in the focal population, often with an emphasis on putatively adaptive (beneficial) characters. Statistical genetics can screen the genome for these variants on the basis of their co-inheritance with phenotype, as they recombine with ancestral alleles in the wild or in laboratory crosses. Elegant validated examples in the recent literature include skeletal shape in populations of stickleback fish (*Gasterosteus aculeatus*) [3, 4], *Drosophila* courtship song [5] and copulatory organs [6], pigmentation in deer mice (*Peromyscus maniculatus*) [7], and herbivore resistance compounds in the plant *Boechera stricta* [8]. From this line of research a number of key principles have emerged, including the role for *cis*-regulatory changes alongside amino acid variants in evolution [9]; the molecular bases of convergent evolution [10]; and the potential for even the most complex traits to arise under neutral forces rather than natural selection [11, 12].

The so-called local adaptation scenario, in which we catch evolution in the act within a species, is actually fairly special. Oftentimes, a modern-day species may not comprise a range of interfertile populations, each with its own niche and adaptive peculiarities. Instead, a given population, as it has become restricted to its new environment, will have picked up mutations that block its ability to interbreed with other relatives. To study the genetics of any of its characters, we would need to make a comparison to another species from some other niche, which lacks the phenotype of interest. But by definition, reproductively isolated species cannot interbreed, so we could never analyze recombinants from a mating between them. Linkage analysis and GWAS, then, are off the table for investigating trait differences between species. Without them, how do we understand the mechanisms of evolutionary innovation in these systems?

As one possible way forward, we could imagine studying a case where, long after an advantageous trait has swept through a species, a deleterious mutation arises in a few individuals, compromising their phenotype. Given recombinants between the latter and other wild-type members of the species, we could deploy GWAS or linkage mapping. We'd then find the deleterious allele as it erodes function in these few defective isolates. The locus, however, doesn't necessarily shed light on the alleles that evolution used to build the trait in the first place. The distinction is a familiar one from classical genetics: studying recent losses of a phenotype will reveal genes that are *necessary* for the species to maintain the status quo, whereas we started out wanting to know which elements would have been *sufficient* to gain the trait in an ancestral background. To find the latter in interspecies comparisons, we must make use of different tools.

Comparative genomics between species

To date, what we know about the genetics of traits as they distinguish different species has come mostly from two lines of investigation. One paradigm simply rests on a well-informed guess. When model-organism studies build up a body of knowledge about the function of a gene, it can be a good bet to test for a functional impact of differences at the locus across taxa. Among the recent successes of this candidate-gene approach are validated discoveries of determinants of species-specific pigmentation in *Dario* (zebrafish) [13]; DNA repair in Proboscidea (elephants) [14] and *Castor canadensis* (beavers) [15]; interferon response in *Rhinolophus sinicus*, *Myotis davidii*, and *Pteropus alecto* (bats) [16]; and flightlessness in ratites (ostriches, kiwi, *etc.*) [17]. Another approach to interspecies genetics uses profiles of molecular characters—like DNA sequence variants, expression programs, or epigenetic patterns—that distinguish a given species from sister taxa. Comparative-genomic data of this kind has led recently to satisfying validated reports of variant loci governing scales in *Phoxinellus* fish [18]; fatty acid metabolism in stickleback [19]; host defense in humans and mice [20]; sweet taste in *Calypte anna* (Anna's hummingbirds) [21]; and host specialization by *Phytophthora mirabilis* (potato blight) [22].

Against the backdrop of these landmark studies, it's important to note that we expect most traits to be genetically complex, *i.e.* the product of variants at multiple independent loci in the genome. Validation follow-up to a candidate-based or comparative-genomic study will usually emphasize a single major determinant of the phenotype of interest. The complex genetic architecture of the trait will rarely get determined.

We would summarize the traditional state of the field, then, as a Catch-22 dilemma for any geneticist interested in evolutionary innovation. Trait variation within a species can be fairly readily dissected in an unbiased manner by GWAS or linkage analysis, but only in rare cases can we track down an evolutionary innovation for this purpose as it emerges in a population. By contrast, conserved innovation traits that define a species are easier to spot in the wild, but their genetics have been inaccessible to genome-scale experimental mapping.

New methods for interspecies genetics

We could break through this impasse, and learn a lot more about the mechanisms of evolutionary innovation, if it were easier to map genotype to phenotype in an unbiased way between species. Recently, new experimental methods have come forward with this goal in mind. The idea is to run a genome-scale phenotypic screen across a panel of synthetic strains, each of which is a mosaic of inheritance from two genetically distinct parents but generated without the activation of a meiotic program. With this advance, we no longer need to restrict mapping analyses to closely related, reproductively compatible lines. Statistical genetics can cover more diversity than ever before.

Mitotic recombination.

Two new unbiased methods to dissect natural trait variation use mitotic recombination to mix up the genomes of a pair of genetically distinct parent lines (Figure 1A). For each strategy, the starting point is a stable hybrid formed by mating the two parents. In one report,

crossovers were induced along one chromosome of a yeast hybrid via a targeted library of CRISPR-Cas9-targeted double-strand breaks [23], a precise though potentially costly approach. In the other new strategy, inhibition of the BLM helicase (a recombination repressor) by the drug ML216 induced untargeted recombinations across the genome in mouse hybrid stem cells [24]. These treatments each yielded a cohort of recombinant progeny for sequencing and phenotyping. Then, in each case an *in silico* scan detected genetic variants co-inherited with (linked to) the trait of interest among the progeny—manganese and 6-thioguanine resistance, in the yeast and mouse work respectively.

The methods are so new that they have not yet advanced beyond proof of concept studies in cell culture. How would they work in whole multicellular organisms or organs? For systems in which stem cell techniques can be used (as in [24]), hybrid stem cells would be subjected to induced recombination, then either differentiated into a cell type or organoid of interest, or grown to maturity. As in the pilot papers, phenotyping and sequencing at scale would then enable linkage mapping. Ideally, any such study would find a way to beat the problem of low genetic resolution: variants near one another on the chromosome will show the same degree of linkage signal if they are not broken up by recombination. In other words, one can't distinguish a causal locus underlying the trait from neighboring sites that are passed down with it from parent to offspring. The published studies in cells achieved tight resolution by using deep guide RNA libraries in the Cas9 approach [23] and large populations for the ML216 method [24].

Reciprocal hemizygosity.

A complementary strategy from our own group, RH-seq [25] (Figure 1B), uses a scheme called the reciprocal hemizygosity test [26–28]. In a hybrid from the mating of two divergent parent lines, we generate hemizygote clones harboring random insertional mutations. Our pilot project used a transposon for this purpose. In each clone the allele from one of the two parents at a single site in the diploid hybrid genome is disrupted, revealing the function of the other, uncovered allele. After bulk phenotyping and sequencing, for a focal gene we collate the phenotypes of all clones harboring mutations in each parent's allele in turn, which are effectively replicates of each other. Any difference between these two suites of phenotypes—one for each allele—reveals the impact on phenotype of DNA sequence variants at the locus. Our pilot publication describing RH-seq [25] centered on an adaptive thermotolerance trait distinguishing one yeast from reproductively isolated sister species.

How can we apply RH-seq to multicellular organisms, say in a comparison between two divergent taxa? In one scheme, a practitioner of the method would mutagenize gametes of each parent line in turn, then mate to a wild-type of the other line and collect whole-organism hemizygotes for screening (Figure 1B, top). This could have particular appeal for invertebrate animals and plants, including many non-model systems, as it doesn't rely on expensive or sophisticated molecular tools. RH-seq could also lend itself well to stem cells, when they can be mutagenized and differentiated into organoids or developing embryos to be screened (Figure 1B, bottom). Note, however, that for the latter setup in cultured cells, the reciprocal hemizygosity test won't make sense for a sex chromosome, in any species in which one copy is silenced in the homogametic sex. In either implementation, RH-seq will

map the determinants of a trait of interest to single-gene resolution, but needs large hemizygote pools to achieve it—so the phenotyping must be high-throughput.

Genome-wide allele replacement.

Another new approach directly tests the impact of natural variation between two divergent parents by swapping, at each variant site, the allele from one background into the other [29] (Figure 1C). The genomic panel of targeted allelic replacement lines is generated with a library of retron-encoded CRISPR-Cas9 machinery called CRISPEY, and screened and sequenced in bulk. The proof of concept publication used yeast growth in minimal medium as a trait [29].

The CRISPEY method, like the others we have discussed, could port to multicellular organisms in which stem cell techniques are available: the progenitors would be edited with a genome-wide library of guide RNAs and then differentiated for screening. The most rigorous such implementation would generate several redundant allele-swap clones per variant for reproducibility's sake, though this adds to the (perhaps already appreciable) scale and cost. As an interesting distinction from the other strategies we have covered here, only the CRISPEY approach does genetics in a purebred background. Strong epistasis could erode its power, for example in a suite of coevolved adaptive loci that depend on one another for function. That is, transgenesis of each allele on its own into a foreign background may not lead to detectable phenotypic effects. However, for additively acting loci, allele replacement in a purebred context is the gold standard test for a relationship between genotype and phenotype.

A focus on long-term evolutionary innovation

Together, the methods outlined in Figure 1 help position the field for unbiased genetic mapping between divergent species. Not too divergent, however—all except the CRISPEY approach require phenotyping in hybrids. This constraint rules out any lineages that cannot mate, or that confer karyotypic instability or other unusual phenotypes once they come together. That said, for any choice of method, we will likely make the most headway when we target the closely related members of species complexes. These species will often have roughly the same body plan and metabolic systems. We can expect that evolution used tractably few genes to build a particular trait that differs between them, and we can hope to find these loci.

The latter logic might sound familiar, in that it echoes our description above of genetic mapping in recently diverged populations that can still interbreed. What's different, in a focus on species-level variation, is that we can pry a bit farther open the window of timescales on which to do genetics. If a lineage has been diverging long enough to become reproductively isolated, it may well have refined a trait over that whole span of time. A given phenotypic difference between extant species could thus have fairly ancient origins, and in many cases we can now tell its genetic story.

Concluding remarks and future perspectives

The unbiased dissection of ancient traits will help solve a number of mysteries in evolutionary biology (see Outstanding Questions). For one, we know surprisingly little about the mechanisms of long-term evolution. We assume that many complex phenotypes have been built over millions of years, *e.g.* by the processes of natural selection—but how exactly? A few clues have come to light from experimental evolution [1, 30, 31] and ancestral reconstruction of model proteins [32–36]. This literature paints a picture in which a population undergoes multiple waves of innovation, each depending on the background set up by its predecessor. Now geneticists can venture into the wild and test such a model more fully, using the tools for interspecies mapping that we have covered here.

As another open question in the field, consider the process of speciation. We know that organisms specializing to a new niche tend to lose the ability to reproduce with the population they left behind. We don't know whether the molecular mechanisms are intertwined. In a theoretical scenario called a magic trait [37], the alleles underlying an adaptation in a species are the very loci that also govern its infertility with relatives maintaining the ancestral program. Evidence for this model has cropped up in experimental evolution studies [38, 39], but we haven't yet established whether and how it happens in the wild. The answers will be revealed as interspecies genetics takes off.

Alongside these benefits to the study of basic evolutionary biology, the new genetic methods will also help the field fulfill its translational potential. As we have discussed, a given trait we know from the natural world as a feat of bioengineering—including spiders' silk and mole rat longevity—will often be tightly conserved in its respective species. It's only by contrast with an evolutionary neighbor that we can get perspective on the underlying genes, with the goal of using them for good in an industrial setting. And the unbiased search for these loci is now coming within reach, even across reproductive barriers.

Acknowledgements

The authors thank Herb Kasler and members of the Brem lab for helpful discussions. This work was supported by NIH R01 GM120430-A1 and a Glenn Award for Research in Biological Mechanisms of Aging to R.B.B.

Glossary

***Cis*-regulatory element**
Non-coding section of the genome that regulates neighboring gene(s)

Epistasis
The tendency for the impact of allelic variation at one locus to depend on inheritance at another locus

Genetic architecture
All the genetic determinants of a naturally varying trait and the mechanisms by which they contribute

Genetic resolution

In a linkage or association study, the degree to which the phenotypic effects of DNA sequence variants close to one another on the chromosome can be distinguished

Genome-wide association study (GWAS)
A strategy to elucidate the genetic basis of variation in a phenotype across unrelated individuals of a given species, by testing each DNA sequence variant in turn for a pattern of co-inheritance with the trait of interest

Genome-scale phenotypic screen
Any experimental method to determine the genetic basis of a phenotype that scans the entire genome in an unbiased manner

Hemizygote
A diploid in which a portion of a chromosome is present in only one copy

Homogametic sex
In species whose sexes are determined by sex chromosomes, the sex of an individual in which both sex chromosomes are the same

Interfertile
Capable of mating to form non-sterile progeny

Linkage mapping
A strategy to elucidate the genetic basis of variation in a phenotype across individuals in a family, by testing each DNA sequence variant in turn for a pattern of co-inheritance with the trait of interest

Reciprocal hemizygosity test
A test for the impact on a phenotype of allelic variation between two genetically distinct parents, by comparing the phenotype of diploid hybrid clones harboring a disrupting mutation in each allele in turn

Retron
A natural DNA element encoding reverse transcriptase and a single-stranded DNA template

REFERENCES

1. Savolainen O. et al. (2013) Ecological genomics of local adaptation. Nat Rev Genet 14 (11), 807–20. [PubMed: 24136507]
2. Nadeau NJ and Jiggins CD. (2010) A golden age for evolutionary genetics? Genomic studies of adaptation in natural populations. Trends Genet 26 (11), 484–92. [PubMed: 20851493]
3. Peichel CL and Marques DA. (2017) The genetic and molecular architecture of phenotypic diversity in sticklebacks. Philos Trans R Soc Lond B Biol Sci 372 (1713).
4. Cleves PA. et al. (2018) An intronic enhancer of Bmp6 underlies evolved tooth gain in sticklebacks. PLoS Genet 14 (6), e1007449. [PubMed: 29902209]
5. Ding Y. et al. (2016) Natural courtship song variation caused by an intronic retroelement in an ion channel gene. Nature 536 (7616), 329–32. [PubMed: 27509856]
6. Nagy O. et al. (2018) Correlated Evolution of Two Copulatory Organs via a Single cis-Regulatory Nucleotide Change. Curr Biol 28 (21), 3450–3457 e13. [PubMed: 30344115]

7. Linnen CR. et al. (2013) Adaptive evolution of multiple traits through multiple mutations at a single gene. Science 339 (6125), 1312–6. [PubMed: 23493712]
8. Prasad KV. et al. (2012) A gain-of-function polymorphism controlling complex traits and fitness in nature. Science 337 (6098), 1081–4. [PubMed: 22936775]
9. Wray GA. (2007) The evolutionary significance of cis-regulatory mutations. Nat Rev Genet 8 (3), 206–16. [PubMed: 17304246]
10. Martin A and Orgogozo V. (2013) The Loci of repeated evolution: a catalog of genetic hotspots of phenotypic variation. Evolution 67 (5), 1235–50. [PubMed: 23617905]
11. Lynch M. (2007) The frailty of adaptive hypotheses for the origins of organismal complexity. Proc Natl Acad Sci U S A 104 Suppl 1, 8597–604. [PubMed: 17494740]
12. Anderson EC and Slatkin M. (2003) Orr's quantitative trait loci sign test under conditions of trait ascertainment. Genetics 165 (1), 445–6. [PubMed: 14575044]
13. Spiewak JE. et al. (2018) Evolution of Endothelin signaling and diversification of adult pigment pattern in Danio fishes. PLoS Genet 14 (9), e1007538. [PubMed: 30226839]
14. Sulak M. et al. (2016) TP53 copy number expansion is associated with the evolution of increased body size and an enhanced DNA damage response in elephants. Elife 5.
15. Tian X. et al. (2019) SIRT6 Is Responsible for More Efficient DNA Double-Strand Break Repair in Long-Lived Species. Cell 177 (3), 622–638 e22. [PubMed: 31002797]
16. Xie J. et al. (2018) Dampened STING-Dependent Interferon Activation in Bats. Cell Host Microbe 23 (3), 297–301 e4. [PubMed: 29478775]
17. Sackton TB. et al. (2019) Convergent regulatory evolution and loss of flight in paleognathous birds. Science 364 (6435), 74–78. [PubMed: 30948549]
18. Daane JM. et al. (2016) Parallelism and Epistasis in Skeletal Evolution Identified through Use of Phylogenomic Mapping Strategies. Mol Biol Evol 33 (1), 162–73. [PubMed: 26452532]
19. Ishikawa A. et al. (2019) A key metabolic gene for recurrent freshwater colonization and radiation in fishes. Science 364 (6443), 886–889. [PubMed: 31147520]
20. Daugherty MD. et al. (2016) Evolution-guided functional analyses reveal diverse antiviral specificities encoded by IFIT1 genes in mammals. Elife 5.
21. Baldwin MW. et al. (2014) Sensory biology. Evolution of sweet taste perception in hummingbirds by transformation of the ancestral umami receptor. Science 345 (6199), 929–33. [PubMed: 25146290]
22. Dong S. et al. (2014) Effector specialization in a lineage of the Irish potato famine pathogen. Science 343 (6170), 552–5. [PubMed: 24482481]
23. Sadhu MJ. et al. (2016) CRISPR-directed mitotic recombination enables genetic mapping without crosses. Science 352 (6289), 1113–6. [PubMed: 27230379]
24. Lazzarano S. et al. (2018) Genetic mapping of species differences via in vitro crosses in mouse embryonic stem cells. Proc Natl Acad Sci U S A 115 (14), 3680–3685. [PubMed: 29563231]
25. Weiss CV. et al. (2018) Genetic dissection of interspecific differences in yeast thermotolerance. Nat Genet 50 (11), 1501–1504. [PubMed: 30297967]
26. Steinmetz LM. et al. (2002) Dissecting the architecture of a quantitative trait locus in yeast. Nature 416 (6878), 326–30. [PubMed: 11907579]
27. Stern DL. (1998) A role of Ultrabithorax in morphological differences between Drosophila species. Nature 396 (6710), 463–6. [PubMed: 9853753]
28. Stern DL. (2014) Identification of loci that cause phenotypic variation in diverse species with the reciprocal hemizygosity test. Trends Genet 30 (12), 547–54. [PubMed: 25278102]
29. Sharon E. et al. (2018) Functional Genetic Variants Revealed by Massively Parallel Precise Genome Editing. Cell 175 (2), 544–557 e16. [PubMed: 30245013]
30. Good BH. et al. (2017) The dynamics of molecular evolution over 60,000 generations. Nature.
31. Toprak E. et al. (2011) Evolutionary paths to antibiotic resistance under dynamically sustained drug selection. Nat Genet 44 (1), 101–5. [PubMed: 22179135]
32. Anderson DP. et al. (2016) Evolution of an ancient protein function involved in organized multicellularity in animals. Elife 5, e10147. [PubMed: 26740169]

33. Anderson DW. et al. (2015) Intermolecular epistasis shaped the function and evolution of an ancient transcription factor and its DNA binding sites. Elife 4, e07864. [PubMed: 26076233]
34. Bridgham JT. et al. (2009) An epistatic ratchet constrains the direction of glucocorticoid receptor evolution. Nature 461 (7263), 515–9. [PubMed: 19779450]
35. Finnigan GC. et al. (2012) Evolution of increased complexity in a molecular machine. Nature 481 (7381), 360–4. [PubMed: 22230956]
36. Liu Q. et al. (2018) Ancient mechanisms for the evolution of the bicoid homeodomain's function in fly development. Elife 7.
37. Servedio MR. et al. (2011) Magic traits in speciation: 'magic' but not rare? Trends Ecol Evol 26 (8), 389–97. [PubMed: 21592615]
38. Meyer JR. et al. (2016) Ecological speciation of bacteriophage lambda in allopatry and sympatry. Science 354 (6317), 1301–1304. [PubMed: 27884940]
39. Castillo DM. et al. (2015) Experimental evolution: Assortative mating and sexual selection, independent of local adaptation, lead to reproductive isolation in the nematode Caenorhabditis remanei. Evolution 69 (12), 3141–55. [PubMed: 26542312]

OUTSTANDING QUESTIONS

- What kinds of genes and variants does evolution use to build traits over millions of years?
- When long-term evolutionary innovation involves multiple loci (*i.e.* is genetically complex), do their effects rely on one another?
- Likewise, in complex trait evolution, do some intermediate combinations of variants lead to fitness defects, implying that they must accumulate in a particular order?
- When an ecologically relevant trait differs between species, do the causal loci also govern the failure of the species to interbreed (the magic trait model)?

HIGHLIGHTS

- Classically, genes underlying natural trait variation have been mapped by screening recombinant progeny from crosses. This only works for interfertile members of a given species, not for comparisons between species.
- We detail the use of new genetic tricks other than natural meiosis to shuffle the genomes of two parents, putting unbiased, interspecific genetic mapping within reach.
- Proof of concept for the new approaches has used yeast and mouse cell models, revealing the mechanisms of ancient divergences that would be inaccessible by other means.
- The new tools for interspecies genetics have the potential for applicability to tissue, organoid, and whole-organism studies.

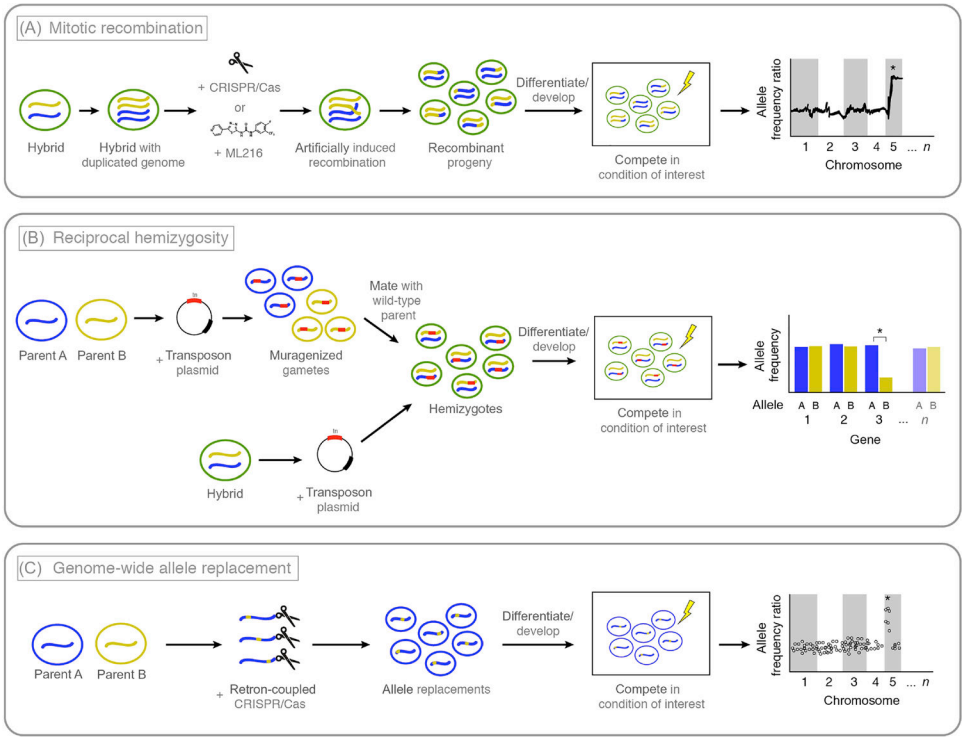

Figure 1. New techniques for genome-wide dissection of trait divergence between species.

Each panel schematizes a method to scan the genome for the impact of allelic variation between divergent parents (yellow and blue). Shown in each case is an example scenario in which one parent's allele at a locus confers improved performance in a phenotypic screen (lightning bolt), and predominates in organismal pools after selection, as detected by sequencing (asterisk, far right). (A) In a hybrid (green) formed from a mating of the two parents, mitotic recombination and loss of heterozygosity are induced on a genomic scale by double-strand breaks directed by CRISPR-Cas9 [23] or inhibition of the recombination repressor BLM by the drug ML216 [24]. If the hybrid is a stem cell, differentiation or development yields cells, organoids, or whole organisms (center). Homozygotes for the well-performing allele are detected at high frequency after phenotypic selection. (B) Untargeted insertional mutagenesis (*e.g.* with a transposon) of the gametes of one parent followed by mating to a wild-type of the other parent (top), or of a stem cell or zygote hybrid (bottom), yields a pool of hemizygotes. In a comparison of mutants at any one locus, those that retain a wild-type copy of the well-functioning allele are detected at high frequency after phenotypic selection [25]. (C) In clones of one parental background (blue), a genome's worth of double-strand breaks directed by CRISPR-Cas9 are each repaired with template from the other parent (yellow). The resulting transgenics, if in a stem cell or zygote, are differentiated or grown to maturity. Those that harbor the well-performing allele are detected at high frequency after phenotypic selection [29].