spleen and bone marrow compartments following secondary and boost immunizations with the model antigen tetanus toxoid, even though the serum level of anti-tetanus toxoid IgG antibody persists and remains elevated for a period of time that exceeds the known half-life of mouse IgG. This result raises the provocative possibility that a sizable fraction of circulating antibodies may be produced by a reservoir of ASCs residing in other anatomical compartments.

The authors also find that the secretion rates of antibodies from ostensibly homogenous ASCs can vary by several orders of magnitude. It is unlikely that such vast differences in protein synthesis are due solely to stochastic effects and transcriptional noise[5]. Could it be that unknown developmental mechanisms or external signals program antibody secretion rates from long-lived plasma cells, and if so, what is the function of such a stratification of antibody production? It is noteworthy that secretion rates do not seem to correlate with antibody affinity, suggesting that selection for high affinity and high expression may be uncoupled.

Yet another interesting observation by Eyer *et al.*[1] is that following immunization, a significant percentage of ASCs secrete IgGs that bind antigens other than the evoking tetanus toxoid immunogen, reminiscent of a previously described hypothesis regarding polyclonal bystander activation of human memory B cells. It would be of interest to determine whether the degree of cross-activation reflects an intrinsic difference between "short-term serological memory" and "long-term serological memory"[6].

As with every new technology, DropMap at present relies on custom-made equipment and demands considerable expertise. However, given the great interest in single-cell analysis tools, the system is likely to be commercialized. As it becomes more widely available, DropMap should facilitate the study of a range of questions of fundamental interest for vaccinology and for the understanding of serological memory. The unique insight provided by tracing single-cell trajectories may help parse the underlying causes of ASC heterogeneity. Is the observed variability in antigen specificity and antibody secretion dynamics a result of other immune cell subsets or of gradations in immune cell function at a single-cell level due to factors such as age and senescence?

Collectively, the results of Eyer *et al.*[1] underscore the differential contributions of ASCs to the serological repertoire, with some ASCs contributing little to the IgG pool. The sheer number and tissue distribution of ASCs at hard-to-access anatomical sites, sampling size limitations, the complex kinetics of antibody secretion, and the long circulatory half-life of antibodies make it impossible to infer the polyclonal serum response to an antigen from single-cell measurements, at least for the foreseeable future. Recent advances in liquid chromatography–tandem mass spectrometry proteomics coupled with B-cell-receptor sequencing now enable the quantification and identification of the monoclonal antibodies in the serum antibody repertoire[7,8]. The combination of serum proteomics and DropMap-based phenotypic analysis of ASCs could be a powerful strategy to investigate the key aspects of ASC phenotypic diversity that shape antibody-mediated immunity.

While Eyer *et al.*[1] only report the analysis of ASCs expressing IgG containing a κ light chain (which account for >95% of antibodies in mice), for human studies it will be necessary to analyze both the λ and κ repertoires, which are distinctly encoded and tend toward equivalent expression in humans. Ultimately, it will be important to integrate the phenotypic data generated using DropMap with transcriptional data on single ASCs[9]. Techniques for single-cell transcriptional analysis with droplet microfluidics are advancing rapidly[10], but combining the complex technologies of DropMap and single-cell transcriptomics is still likely to be quite challenging. Nonetheless, the benefits would be truly transformative. Antibody affinity, antigen selectivity, and sequencing from single cells could be analyzed at an unprecedented scale. Transcriptional profiling of ASCs independent of cell-surface phenotype may identify novel ASC subsets based solely on a molecular definition of the genes they transcribe and the antibodies they secrete. And insights from transcriptomic analyses may provide a more complete understanding of the basis of the secretion heterogeneity observed by Eyer *et al.*[1].

COMPETING FINANCIAL INTERESTS
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Genetics of a hardy crop

Ksenia V Krasileva

New genomic resources for pearl millet are revealing how crops stand up to challenging environments.

Few domesticated plants can thrive in harsh environments. Pearl millet (*Cenchrus americanus* (L.) Morrone) is one of them. It can be cultivated in drought conditions, high salinity and high pH soils, and is a staple in many regions of the world, including Africa and India[1]. Understanding the biological underpinnings of this success could have broad implications for advancing agriculture in regions where improved varieties are most needed. In this issue, Varshney *et al.*[2] report the draft genome sequence of pearl millet and investigate the genetic diversity among 994 pearl millet accessions. In helping to elucidate how pearl millet withstands difficult environmental conditions, the work will guide the improvement of this and other crops, including the development of approaches for adaptation to global climate change.

For genomics tools to be useful in crop improvement, three layers of information are needed: a contiguous reference genome and its annotation; understanding of the genotypic and phenotypic diversity within the species; and predictive models that link genotypes with phenotypes. Varshney *et al.*[2] provide all of these components, including a nearly complete draft assembly of the 1.8-Gb genome, 38,579 annotated proteins, 963 resequenced inbred lines and 31 wild accessions, along with their phenotypes under drought and irrigated conditions. The work brings genomics tools for pearl millet in line with those available for other crops[2].

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Starting with their data, the authors carried out genome-wide association studies to uncover genomic regions linked to beneficial traits. They were able to identify significant markers linked to 15 traits that enhance yield, such as grain size and grain number. These findings can be applied directly in a millet breeding program to derive better crop varieties.

Modern agriculture relies heavily on discovering beneficial effects of combining diverse genotypes, an effect known as hybrid vigor. Information on the genetic differences within species can be a powerful tool to predict and track beneficial combinations. The process of genomic selection, a type of genetic-marker-assisted selection, takes advantage of millions of polymorphisms along the genome that can be analyzed in order to reveal markers linked to beneficial traits[3]. This means that a breeder can pre-screen for beneficial genetic combinations using DNA extracted from small seedlings and predict performance without growing all plants to maturity.

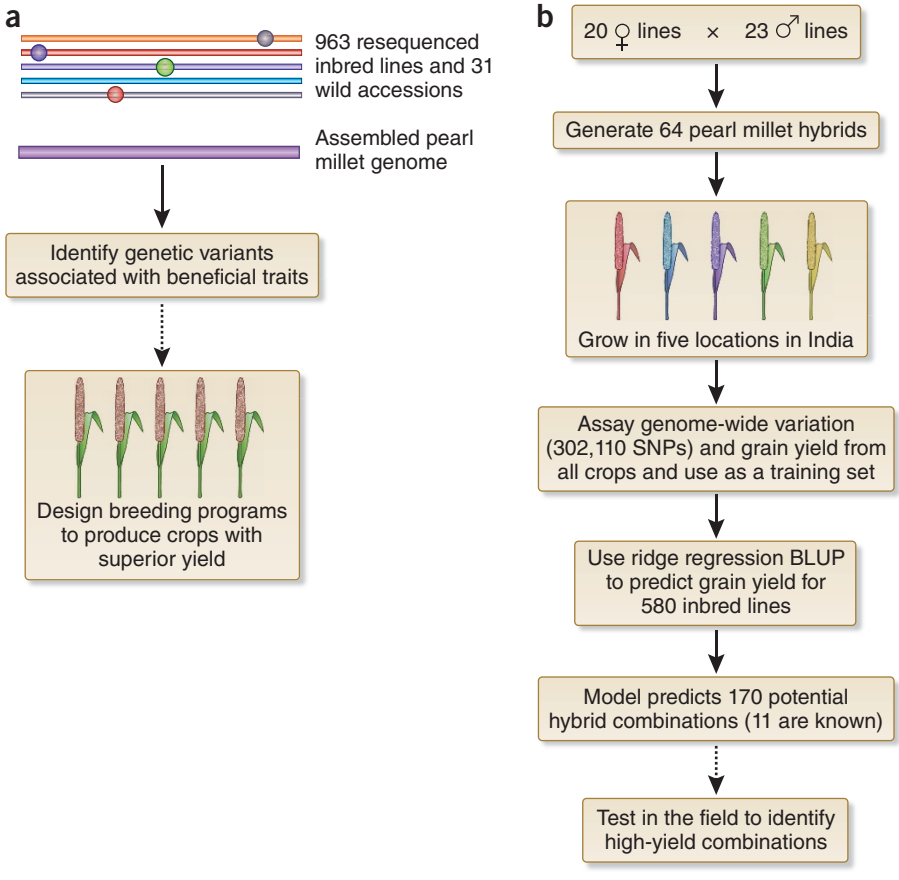
Varshney *et al.*[2] developed genomic selection models to accurately predict hybrid performance under normal and stressed environments (**Fig. 1**). They found 170 promising hybrid combinations: 11 have been used previously and showed better hybrid performance, while 159 combinations have never been tested before. These leads will save breeders time in choosing which crosses to test. For reference, all 963 inbred lines that were sequenced by Varshney *et al.*[2] can be theoretically crossed 464,166 different ways, and twice this number when one accounts for which line is used as a male versus a female parent. The new work thus allows the search space to be reduced by well over 1,000-fold (159 combinations rather than 464,166 × 2).

Comparing the genome sequence of pearl millet, which can withstand heat and thrive in sandy soils, with the genomes of its more vulnerable relatives, such as maize, rice and wheat, is likely to yield valuable insights. Varshney *et al.*[2] looked for expansions of protein families in pearl millet compared to other grasses and identified gene families specifically expanded in millet. Most of these are involved in maintaining a waxy water-repellent plant cuticle called cutin, which correlates with millet's superior drought resilience.

Inclusion of the wild relatives of millet in the analyses is important because their genomes carry beneficial characteristics that are sometimes lost during domestication. Wild relatives often have reservoirs of disease-resistance genes, which can be rapidly identified using targeted gene capture and other next-generation sequencing technologies[4]. Now that nearly 1,000 pearl millet genomes are available, phenotypes that were not reported by Varshney *et al.*[2], such as disease resistance, can be studied on the same germplasm and overlaid on the existing data.

The factors that have restricted cultivation of pearl millet historically are slow generation of new varieties, lack of access to fertilizers and yield losses to weeds, diseases and pests. While pearl millet can grow on sandy and rocky soils that would be too dry for sorghum and maize, the modern sorghum and maize hybrids outperform pearl millet under optimal conditions. Sequencing the millet genome and its biodiversity will allow millet to be improved to produce better yields and therefore be a more reliable staple for the regions that need it the most. More generally, understanding how this crop thrives in harsh environments should aid efforts to generate other crops with enhanced resistance to heat and various stressors.

Linking genomics with biodiversity can accelerate the next agricultural revolution. The key challenge is translating the resulting discoveries into improved varieties and distributing the seeds to farmers who need them most. About 70% of people in Africa and ~80% of the continent's poor live in rural areas[5]. Providing these areas with improved seeds requires the coordinated efforts of researchers, breeders and governments. So far, the most notable projects aiming to achieve this are the Drought Tolerant Maize for Africa Project (http://dtma.cimmyt.org/) and the Next Generation Cassava Breeding Project (http://www.nextgencassava.org/). The Drought Tolerant Maize for Africa[6] and its subsequent seed scaling projects have been run by the International Wheat and Maize Improvement Centre and have already contributed drought-tolerant, well-adapted maize hybrids to help farmers across 13 countries in eastern, west and southern Africa, benefitting



Dibbie Maizels/Springer Nature

Figure 1 Using the pearl millet genome for crop improvement. (**a**) Genome-wide association studies identified genetic variants associated with 15 traits that enhance yield. These findings may be used in breeding programs to produce crops with superior yield. (**b**) Prediction of hybrid performance. Grain yield was measured for 64 pearl millet hybrids grown in five different environments. 302,110 single-nucleotide polymorphisms (SNPs) were assayed across the hybrids. The genotypic and phenotypic data from this experiment were used to train a ridge regression best linear unbiased prediction (BLUP) method, which was used to predict hybrid combinations with high grain yield. Eleven of the predicted combinations are already known to produce hybrids with superior performance. The model predicted 159 combinations that may be promising candidates for developing new high-yield hybrids.

an estimated 43 million people. The Next Generation Cassava Project is implementing genomic selection to systematically improve the yield and nutritional value of this African staple. As modern techniques are brought to bear on other crops, such as yams and millets, they will continue to enrich the world's table.

COMPETING FINANCIAL INTERESTS
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Mosaic mice ace functional genomics

Understanding the function of a gene ultimately requires experiments in the native tissue environment where it is expressed. In mammals, however, the technologies available for such studies are often limited. Writing in *Cell*, Benedito and colleagues[1] present ifgMosaic, a new set of tools that finally allows mouse geneticists to efficiently create the kinds of genetic mosaics that have long been indispensable to fly researchers. "This fills a real gap. We need to be able to do more clonal analysis in mice," says Bon-Kyoung Koo, a stem cell biologist at the Institute of Molecular Biotechnology in Vienna.

In a mosaic animal, different sets of cells carry different genetic material. This is an ideal system for analyzing gene function since the phenotypes of various cell populations can be compared in the same tissue without other confounding factors. In flies, mosaics are readily created by mitotic recombination, but developing similar techniques for mice has proved challenging. Previous methods have been hampered by a low percentage of modified cell clones or unreliable coexpression of reporter genes.

The method of Benedito and colleagues[1] exploits the mutually exclusive *loxP* sites used for combinatorial labeling of neurons in the Brainbow or Confetti mice popular in neuroscience. Their standard constructs consist of an array of fluorescent markers separated by three different *loxP* sites. Recombination by tamoxifen-induced Cre recombinase has only three possible outcomes, each leading to exclusive expression of one of the fluorescent markers. ifgMosaic constructs come in two flavors, allowing for easy analysis of cell numbers and morphology: iMb-Mosaic, with membrane-bound fluorescent proteins, and iChr-Mosaic, with chromatin-targeting fluorescent proteins. Each marker can be coupled to the expression of a gene of interest using a 2A peptide for bicistronic expression of marker and gene. iMb and iChr constructs can be combined to study the consequences of combinatorial expression of up to six genes, creating 15 different subpopulations (in addition to unrecombined wild-type cells) that express either one or a combination of two genes in the constructs.

"This strategy is very elegant and takes the basic idea used in the Confetti mice to the next level," says Janet Rossant, a developmental biologist at the Hospital for Sick Children in Toronto. "The combination of nuclear and membrane labels is especially important for downstream analyses."

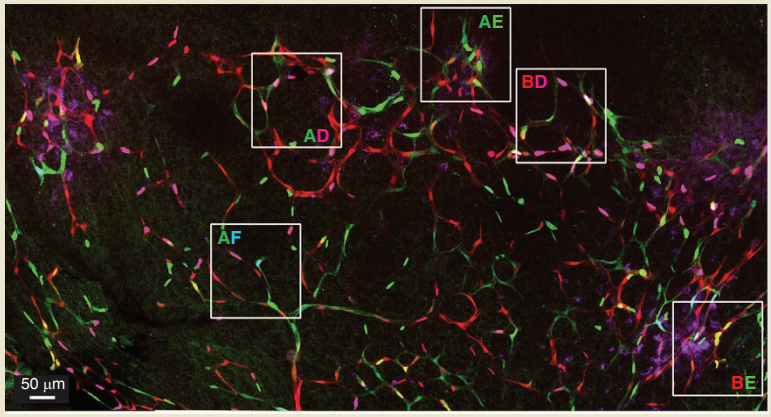

Image taken from Ref. 1

Generating dual ifgMosaic animals does require the integration of a single copy of large multi-gene constructs into the genome—itself not a small feat. To facilitate this process, the authors offer two integration methods. In the first, fertilized eggs are injected with a bacterial artificial chromosome (BAC) containing part of the *Rosa26* locus and isolators to minimize the effects of neighboring regulatory elements and ensure efficient transcription. Random genomic insertion of the BAC is mediated by flanking piggyBac transposon inverted repeats and a co-injected piggyBac transposase. Up to 26% of the resulting pups carry a genomic BAC insertion. Cloning of the expression cassettes with the genes of interest can be done by recombineering in *Escherichia coli*.

Although these BAC constructs worked well in transgenic mice, they performed less well in cell culture. The authors therefore also developed a system to target the ifgMosaic constructs to the endogenous mouse *Rosa26* locus. First, they generated a mouse embryonic stem cell line with a modified *Rosa26* locus containing all the constant elements of the ifgMosaic constructs. The specific elements were then inserted into a unique sequence at the center of the modified locus using CRISPR–Cas9, achieving a 56% integration rate.

"The authors make all these constructs freely available," says Koo. "This will be an enormous help when trying to set up the system, although assembling the necessary constructs for one's own experiments will not be entirely straightforward for some labs. One also has to keep in mind that by its very nature, mosaic analysis can be misleading if the gene of interest works in a non-cell-autonomous manner."

How the constructs can be used is highlighted by the authors' analysis of the role of Notch and VEGF pathways in neurogenesis and angiogenesis in mouse embryos. For example, they express combinations of Notch and VEGF signaling activators and inhibitors, and analyze how clones expressing different combinations proliferate in the same tissue. Endothelial cells with high Notch activity do not benefit from additional VEGF signaling, whereas cells with low VEGF activity proliferate only if Notch activity is also low.

Many extensions of ifgMosaic can be envisioned. "It will be essential to further develop the method to enable gene inactivation, which can probably be done by expressing an shRNA [short hairpin RNA], an sgRNA [single guide RNA] in combination with Cas9, or a dominant negative," says Rossant. "This method can potentially provide such a wealth of information that data analysis will be the next challenge."

Markus Elsner,
Senior Editor

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