Sunday Evening News

Week 16 (2018-04-16 / 04-22)

Selected and edited by ***BGF*** Jany

G-TwYST: **DRAFT CONCLUSIONS AND RECOMMENDATIONS PRESENTED AT THE G-TWYST FINALCONFERENCE**
https://www.g-twyst.eu/files/Conclusions-Recommendations/G-TwYSTConclusionsandrecommendations-draft.pdf

EuropaBio: **EU must reinstate science in GMO safety assessment and eliminate unnecessary animal testing**
http://www.europabio.org/agricultural-biotech/publications/eu-must-reinstate-science-gmo-safety-assessment-and-eliminate?mc_cid=10b9677d1f&mc_eid=b89825d9e5

EuropaBio hat eine sehr objektive Stellungnahme dazu abgegeben und den Aussagen kann ich nur voll zustimmen.

European Risk Forum (ERF): **"SCIENTIFIC INTEGRITY, PUBLIC POLICY, AND BETTER REGULATION' EVENT - INSIGHTS FROM PARTICIPANTS AND THE EUROPEAN RISK FORUM**
http://www.riskforum.eu/uploads/2/5/7/1/25710097/erf_-_pn_32_-_scientific_integrity_event_18.pdf

Smyth S., Martin Phillipson M. - Journal of International Law and Trade Policy
How 'regulatory lag' in approving GMO crops hurts international trade
https://geneticliteracyproject.org/2018/04/20/how-regulatory-lag-in-approving-gmo-crops-hurts-international-trade/ here see also publication 2016

tg – transkript: **BMBF stärkt Biologisierung der Industrie**
Kurz vor dem Weltgipfel der Bioökonomie, dem Global Bioeconomy Summit (19.–20.4.2018) in Berlin, hat Bundesforschungsministerin Anja Karliczek drei Initiativen angekündigt, um die Bioökonomie und die Dynamik innovativer Ausgründungen zu stärken.
https://transkript.de/news/bmbf-staerkt-biologisierung-der-industrie.html

Shukla-Jones, A., S. Friedrichs and D. Winickoff (2018), **"Gene editing in an international context: Scientific, economic and social issues across sectors**", *OECD Science, Technology and Industry Working Papers*, 2018/04, OECD Publishing, Paris
http://dx.doi.org/10.1787/38a54acb-en
https://www.oecd-ilibrary.org/industry-and-services/gene-editing-in-an-international-context_38a54acb-en
https://www.oecd-ilibrary.org/docserver/38a54acb-en.pdf?expires=1524221424&id=id&accname=guest&checksum=8E1BE40B165C1CB28DF7DC4629FE7DFD
pdf-file

Li X. et al. (2018): **Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing**. Front. Plant Sci., doi: 10.3389/fpls.2018.00559
Numerous studies have been focusing on breeding tomato plants with enhanced lycopene accumulation, considering its positive effects of fruits on the visual and functional properties. In this study, we used a bidirectional strategy: promoting the biosynthesis of lycopene, while inhibiting the conversion from lycopene to β- and α-carotene. The accumulation of lycopene was promoted by knocking down some genes associated with the carotenoid metabolic pathway. Finally, five genes were selected to be edited in genome by CRISPR/Cas9 system using Agrobacterium tumefaciens-mediated transformation. Our findings indicated that CRISPR/Cas9 is a site-specific genome editing technology that allows highly efficient target mutagenesis in multiple genes of interest. Surprisingly, the lycopene content in tomato fruit subjected to genome editing was successfully increased to about 5.1-fold. The homozygous mutations were stably transmitted to subsequent generations. Taken together, our results suggest that CRISPR/Cas9 system can be used for significantly improving lycopene content in tomato fruit with advantages such as high efficiency, rare off-target mutations, and stable heredity.
https://www.frontiersin.org/articles/10.3389/fpls.2018.00559/abstract

Haselmair-Gosch C. et al. (2018): **Great Cause—Small Effect: Undeclared Genetically Engineered Orange Petunias Harbor an Inefficient Dihydroflavonol 4-Reductase**. Front. Plant Sci., https://doi.org/10.3389/fpls.2018.00149

A recall campaign for commercial, orange flowering petunia varieties in spring 2017 caused economic losses worldwide. The orange varieties were identified as undeclared genetically engineered (GE)-plants, harboring a maize dihydroflavonol 4-reductase (*DFR, A_1*), which was used in former scientific transgenic breeding attempts to enable formation of orange pelargonidin derivatives from the precursor dihydrokaempferol (DHK) in petunia. How and when the *A_1* cDNA entered the commercial breeding process is unclear. We provide an in-depth analysis of three orange petunia varieties, released by breeders from three countries, with respect to their transgenic construct, transcriptomes, anthocyanin composition, and flavonoid metabolism at the level of selected enzymes and genes. The two possible sources of the *A_1* cDNA in the undeclared GE-petunia can be discriminated by PCR. A special version of the *A_1* gene, the *A_1* type 2 allele, is present, which includes, at the 3'-end, an additional 144 bp segment from the non-viral transposable *Cin4-1* sequence, which does not add any functional advantage with respect to DFR activity. This unequivocally points at the first scientific GE-petunia from the 1980s as the *A_1* source, which is further underpinned e.g., by the presence of specific restriction sites, parts of the untranslated sequences, and the same arrangement of the building blocks of the transformation plasmid used. Surprisingly, however, the GE-petunia cannot be distinguished from native red and blue varieties by their ability to convert DHK in common *in vitro* enzyme assays, as DHK is an inadequate substrate for both the petunia and maize DFR. Recombinant maize DFR underpins the low DHK acceptance, and, thus, the strikingly limited suitability of the *A_1* protein for a transgenic approach for breeding pelargonidin-based flower color. The effect of single amino acid mutations on the substrate specificity of DFRs is demonstrated. Expression of the *A_1* gene is generally lower than the petunia *DFR* expression despite being under the control of the strong, constitutive p*35S* promoter. We show that a rare constellation in flavonoid metabolism—absence or strongly reduced activity of both flavonol synthase and B-ring hydroxylating enzymes—allows pelargonidin formation in the presence of DFRs with poor DHK acceptance.
https://www.frontiersin.org/articles/10.3389/fpls.2018.00149/full pdf-file available

This publication was already disturbed as a preprint:
Wang, X., Liu, Q., Meissle, M., Peng, Y., Wu, K., Romeis, J. and Li, Y. (2018): **Bt rice could provide ecological resistance against nontarget planthoppers.** Plant Biotechnol. J., https://doi.org/10.1111/pbi.12911 pdf-file available

Peng D. et al. (2018**): Enhancing freezing tolerance of *Brassica napus* L. by overexpression of a stearoyl-acyl carrier protein desaturase gene (*SAD*) from *Sapium sebiferum* (L.) Roxb**. Plant Science 272, 32-41; https://doi.org/10.1016/j.plantsci.2018.03.028

Sapium sebiferum (L.) Roxb. is an important woody oil tree and traditional herbal medicine in China. Stearoyl-acyl carrier protein desaturase (SAD) is a dehydrogenase enzyme that plays a key role in the transformation of saturated fatty acids into unsaturated fatty acids in oil; these fatty acids greatly influence the freezing tolerance of plants. However, it remains unclear whether freezing tolerance can be regulated by the expression level of *SsSAD* in *S. sebiferum* L. Our research indicated that *SsSAD* expression in *S. sebiferum* L. increased under freezing stress. To further confirm this result, we constructed a *pEGAD-SsSAD* vector and transformed it into *B. napus* L. *W10* by *Agrobacterium tumefaciens*-mediated transformation. Transgenic plants that overexpressed the *SsSAD* gene exhibited significantly higher linoleic (18:2) and linolenic acid (18:3) content and advanced freezing tolerance. These results suggest that *SsSAD* overexpression in *B. napus* L. can increase the content of polyunsaturated fatty acids (PUFAs) such as linoleic (18:2) and linolenic acid (18:3), which are likely pivotal in improving freezing tolerance in *B. napus* L. plants. Thus, *SsSAD* overexpression could be useful in the production of freeze-tolerant varieties of *B. napus* L.
https://www.sciencedirect.com/science/article/pii/S0168945217310257

Ortega JL, Rajapakse W, Bagga S, Apodaca K, Lucero Y, Sengupta-Gopalan C (2018): **An intragenic approach to confer glyphosate resistance in chile (*Capsicum annuum*) by introducing an *in vitro* mutagenized chile *EPSPS* gene encoding for a glyphosate resistant EPSPS protein.** PLoS ONE 13(4): e0194666. https://doi.org/10.1371/journal.pone.0194666

Chile pepper (*Capsicum annuum*) is an important high valued crop worldwide, and when grown on a large scale has problems with weeds. One important herbicide used is glyphosate. Glyphosate inactivates the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*), a key enzyme in the synthesis of aromatic amino acids. A transgenic approach towards making glyphosate resistant plants, entails introducing copies of a gene encoding for glyphosate-resistant EPSPS enzyme into the plant. The main objective of our work was to use an intragenic approach to confer resistance to glyphosate in chile which would require using only chile genes for transformation including the selectable marker. Tobacco was used as the transgenic system to identify different gene constructs that would allow for the development of the intragenic system for chile, since chile transformation is inefficient. An *EPSPS* gene was isolated from chile and mutagenized to introduce

substitutions that are known to make the encoded enzyme resistant to glyphosate. The promoter for *EPSPS* gene was isolated from chile and the mutagenized chile *EPSPS* cDNA was engineered behind both the *CaMV35S* promoter and the *EPSPS* promoter. The leaves from the transformants were checked for resistance to glyphosate using a cut leaf assay. In tobacco, though both gene constructs exhibited some degree of resistance to glyphosate, the construct with the *CaMV35S* promoter was more effective and as such chile was transformed with this gene construct. The chile transformants showed resistance to low concentrations of glyphosate. Furthermore, preliminary studies showed that the mutated *EPSPS* gene driven by the *CaMV35S* promoter could be used as a selectable marker for transformation. We have shown that an intragenic approach can be used to confer glyphosate-resistance in chile. However, we need a stronger chile promoter and a mutated chile gene that encodes for a more glyphosate resistant EPSPS protein.
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0194666
http://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0194666&type=printable

Niehl, A. et al. (2018): **Synthetic biology approach for plant protection using dsRNA**. Plant Biotechnology J., doi: 10.1111/pbi.12904.
Pathogens induce severe damages on cultivated plants and represent a serious threat to global food security. Emerging strategies for crop protection involve the external treatment of plants with double-stranded (ds)RNA to trigger RNA interference. However, applying this technology in greenhouses and fields depends on dsRNA quality, stability and efficient large-scale production. Using components of the bacteriophage phi6, we engineered a stable and accurate *in vivo* dsRNA production system in *Pseudomonas syringae* bacteria. Unlike other *in vitro* or *in vivo* dsRNA production systems that rely on DNA transcription and postsynthetic alignment of single-stranded RNA molecules, the phi6 system is based on the replication of dsRNA by an RNA-dependent RNA polymerase, thus allowing production of high-quality, long dsRNA molecules. The phi6 replication complex was reprogrammed to multiply dsRNA sequences homologous to tobacco mosaic virus (TMV) by replacing the coding regions within two of the three phi6 genome segments with TMV sequences and introduction of these constructs into *P. syringae* together with the third phi6 segment, which encodes the components of the phi6 replication complex. The stable production of TMV dsRNA was achieved by combining all the three phi6 genome segments and by maintaining the natural dsRNA sizes and sequence elements required for efficient replication and packaging of the segments. The produced TMV-derived dsRNAs inhibited TMV propagation when applied to infected *Nicotiana benthamiana* plants. The established dsRNA production system enables the broad application of dsRNA molecules as an efficient, highly flexible, nontransgenic and environmentally friendly approach for protecting crops against viruses and other pathogens.
https://onlinelibrary.wiley.com/doi/epdf/10.1111/pbi.12904

Theodorou P., Radzevičiūtė R., Kahnt B., Soro A., Grosse I., Robert J. Paxton R.J. (2018) **Genome-wide single nucleotide polymorphism scan suggests adaptation to urbanization in an important pollinator, the red-tailed bumblebee (Bombus lapidarius L.).** *Proceedings of the Royal Society B: Biological Sciences* (2018). DOI: 10.1098/rspb.2017.2806
Urbanization is considered a global threat to biodiversity; the growth of cities results in an increase in impervious surfaces, soil and air pollution, fragmentation of natural vegetation and invasion of non-native species, along with numerous environmental changes, including the heat island phenomenon. The combination of these effects constitutes a challenge for both the survival and persistence of many native species, while also imposing altered selective regimes. Here, using 110 314 single nucleotide polymorphisms generated by restriction-site-associated DNA sequencing, we investigated the genome-wide effects of urbanization on putative neutral and adaptive genomic diversity in a major insect pollinator, *Bombus lapidarius*, collected from nine German cities and nine paired rural sites. Overall, genetic differentiation among sites was low and there was no obvious genome-wide genetic structuring, suggesting the absence of strong effects of urbanization on gene flow. We nevertheless identified several loci under directional selection, a subset of which was associated with urban land use, including the percentage of impervious surface surrounding each sampling site. Overall, our results provide evidence of local adaptation to urbanization in the face of gene flow in a highly mobile insect pollinator.
http://rspb.royalsocietypublishing.org/content/285/1877/20172806

and Martin-Luther-Universität Halle-Wittenberg
Urban life leaves behind traces in the genome of bumblebees
https://phys.org/news/2018-04-urban-life-genome-bumblebees.html#jCp

Katherine Dentzman K. (2018): **"I would say that might be all it is, is hope": The framing of herbicide resistance and how farmers explain their faith in herbicides**. Journal of Rural Studies 57:118-127; https://doi.org/10.1016/j.jrurstud.2017.12.010
pdf-file: https://ac.els-cdn.com/S0743016717303662/1-s2.0-S0743016717303662-main.pdf?_tid=bcd82c1e-ae15-4d7a-b2f3-fbc74ed134da&acdnat=1524231214_6df439e1d511841629433820c39841ac

Weidberg H. and Angelika Amon A. (2018): **MitoCPR—A surveillance pathway that protects mitochondria in response to protein import stress.** *Science* Vol. 360, Issue 6385, eaan4146, DOI: 10.1126/science.aan4146
Mitochondria provide cells with energy and numerous essential metabolites such as lipids, amino acids, iron sulfur clusters, and heme. All mitochondrial functions rely on import of proteins into the organelle because the mitochondrial proteome is almost exclusively encoded by nuclear genes. Given the central importance of mitochondria for cell viability, it is not surprising that cells mount a nuclear response when mitochondrial functions are compromised. These mitochondria-to-nucleus signaling pathways include the mtUPR (mitochondrial unfolded protein response), which triggers expression of mitochondrial chaperones when mitochondrial protein folding is defective, and the UPRam (unfolded protein response activated by mistargeting of proteins) and mPOS (mitochondrial precursor over-accumulation stress) pathways, which reduce translation and induce degradation of unimported proteins in the cytosol when mitochondrial import is impaired. Even though mitochondrial import is central to all mitochondrial functions, no response to protein import defects had been described that protects mitochondria during this stress.
http://science.sciencemag.org/content/360/6385/eaan4146

and Raleigh Mcelvery, Massachusetts Institute of Technology
Scientists discover a pathway that monitors a protein import into mitochondria
https://phys.org/news/2018-04-scientists-pathway-protein-import-mitochondria.html#jCp

Plekhanova E., Nuzhdin S.V., Utkin L.V. and Maria G. Samsonova M. G. (2018): **Prediction of deleterious mutations in coding regions of mammals with Transfer learning.** *Evolutionary Applications* (2018). DOI: 10.1111/eva.12607
The genomes of mammals contain thousands of deleterious mutations. It is important to be able to recognize them with high precision. In conservation biology, the small size of fragmented populations results in accumulation of damaging variants. Preserving animals with less damaged genomes could optimize conservation efforts. In breeding of farm animals, trade-offs between farm performance versus general fitness might be better avoided if deleterious mutations are well classified. In humans, the problem of such a precise classification has been successfully solved, in large part due to large databases of disease-causing mutations. However, this kind of information is very limited for other mammals. Here, we propose to better use information available on human mutations to enable classification of damaging mutations in other mammalian species. Specifically, we apply Transfer learning - machine learning methods, improving small data set for solving a focal problem (recognizing damaging mutations in our companion and farm animals) due to use of much large data sets available for solving a related problem (recognizing damaging mutations in humans). We validate our tools using mouse and dog annotated datasets and obtain significantly better results in companion to the SIFT classifier. Then we apply them to predict deleterious mutations in cattle genome-wide dataset.
https://onlinelibrary.wiley.com/doi/epdf/10.1111/eva.12607

and Peter the Great Saint-Petersburg Polytechnic University
Transfer learning meets livestock genomics
https://phys.org/news/2018-04-livestock-genomics.html#jCp

Yoshida et al. (2018): **A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science* 351, Issue 6278, 1196-1199**; DOI: 10.1126/science.aad6359
Poly(ethylene terephthalate) (PET) is used extensively worldwide in plastic products, and its accumulation in the environment has become a global concern. Because the ability to enzymatically degrade PET has been thought to be limited to a few fungal species, biodegradation is not yet a viable remediation or recycling strategy. By screening natural microbial communities exposed to PET in the environment, we isolated a novel bacterium, *Ideonella sakaiensis* 201-F6, that is able to use PET as its major energy and carbon source. When grown on PET, this strain produces two enzymes capable of hydrolyzing PET and the reaction intermediate, mono(2-hydroxyethyl) terephthalic acid. Both enzymes are required to enzymatically convert PET efficiently into its two environmentally benign monomers, terephthalic acid and ethylene glycol.
http://science.sciencemag.org/content/351/6278/1196/tab-pdf

Harry P. Austin H.P. et al. (2018): **Characterization and engineering of a plastic-degrading aromatic polyesterase.** PNAS, DOI:10.1073/pnas.1718804115
Poly(ethylene terephthalate) (PET) is one of the most abundantly produced synthetic polymers and is accumulating in the environment at a staggering rate as discarded packaging and textiles. The properties that make PET so useful also endow it with an alarming resistance to biodegradation, likely lasting centuries in the environment. Our collective reliance on PET and other plastics means that this buildup will continue unless solutions are found. Recently, a newly discovered bacterium, *Ideonella sakaiensis* 201-F6, was shown to exhibit the rare ability to grow on PET as a major carbon and energy source. Central to its PET biodegradation capability is a secreted PETase (PET-digesting enzyme). Here, we present a 0.92 Å resolution X-ray crystal structure of PETase, which reveals features common to both cutinases and lipases. PETase retains the ancestral

α/β-hydrolase fold but exhibits a more open active-site cleft than homologous cutinases. By narrowing the binding cleft via mutation of two active-site residues to conservedamino acids in cutinases, we surprisingly observe improved PET degradation, suggesting that PETase is not fully optimized for crystalline PET degradation, despite presumably evolving in a PET-rich environment. Additionally, we show that PETase degrades another semiaromatic polyester, polyethylene-2,5-furandicarboxylate (PEF), which is an emerging, bioderived PET replacement with improved barrier properties. In contrast, PETase does not degrade aliphatic polyesters, suggesting that it is generally an aromatic polyesterase. These findings suggest that additional protein engineering to increase PETase performance is realistic and highlight the need for further developments of structure/activity relationships for bio-degradation of synthetic polyesters-
http://www.pnas.org/content/pnas/early/2018/04/16/1718804115.full.pdf

Salk J.J., Schmitt M.W. and Lawrence A. Loeb L.A. (2018): **Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations** NATURE REVIEWS | GENETICS, 19, 269-285 doi: 10.1038/nrg.2017.117
Mutations, the fuel of evolution, are first manifested as rare DNA changes within a population of cells. Although next-generation sequencing (NGS) technologies have revolutionized the study of genomic variation between species and individual organisms, most have limited ability to accurately detect and quantify rare variants among the different genome copies in heterogeneous mixtures of cells or molecules. We describe the technical challenges in characterizing subclonal variants using conventional NGS protocols and the recent development of error correction strategies, both computational and experimental, including consensus sequencing of single DNA molecules. We also highlight major applications for low-frequency mutation detection in science and medicine, describe emerging methodologies and provide our vision for the future of DNA sequencing. *
https://www.nature.com/articles/nrg.2017.117 pdf-file available

Tagungen – Meetings

Deutscher Ethikrat
Des Menschen Würde in unserer Hand. Herausforderungen durch neue Technologien
Mittwoch, 27. Juni 2018, 10:00 bis 18:15 Uhr sowie
Donnerstag, 28. Juni 2018, 09:00 bis 16:00 Uhr
Ellington Hotel Berlin
Nürnberger Straße 50 – 55
10789 Berlin
https://idw-online.de/de/news692702

Wie immer wird für Hinweise und der Zusendung von Publikationen und sonstigen Informationen gedankt. pdf-Dateien können meist direkt aus den links heruntergeladen werden.

Bitte besuchen sie auch die Webseite des Wissenschaftlerkreis Grüne Gentechnik e.V. (WGG): www.wgg-ev.de . Hier finden Sie weitere interessante Informationen.

*As always, I thank you all for hints and for publications. M*ost of the pdf files can be downloaded directly from the links.

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