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Review

"When worlds collide and smuts converge": Tales from the 1st International Ustilago/Smut Convergence

Barry J. Saville[a], Michael H. Perlin[b,*]

[a] Forensic Science Program, Trent University, Peterborough, Canada
[b] Department of Biology, Program on Disease Evolution, University of Louisville, Louisville, KY, USA

ABSTRACT

From the evening of March 12, till dinner on March 13, 2017, the 1st International Ustilago/Smut Convergence took place as a workshop prior to the start of the 29th Fungal Genetics Conference, in Asilomar, California. The overall goals of the meeting were to expand the smut model systems being used and to expand participation by the next generations of scientists with these fungi. These goals were implemented through a combination of emphasis on student and post-doc presentations, mentoring of such individuals, and active recruitment of participation by groups under-represented at such meetings in recent years in the US, especially those from Latin America and other Spanish-speaking countries. Work was presented at the first workshop on *U. maydis*, *Sporosorium reilianum*, *Microbotryum violaceum*, *U. esculenta*, and *Thecaphora thlaspeos*. Students and post-doctoral researchers were encouraged to present their "just-in-time," as-yet-unpublished data, in a safe environment, with the understanding of those attending the meeting that this early access was a privilege not to be taken advantage of. The result was lively and constructive discussion, including a variety of presentations by these young scientists on putative and characterized smut effector proteins, clearly at the forefront of such research, even considering the advances presented later that week at the Fungal Genetics Conference. This review also briefly compares the first meeting with the events of the recent 2nd International Ustilago/Smut Convergence (March 11–12, 2019), which ended with a tribute to Prof. Dr. Regine Kahmann, in honor of her career, and especially for her contributions to the field of smut genetics.

1. Introduction

> "During the Convergence, all of the Nine Realms of Yggdrasil are in alignment. This alignment causes the dimensional boundaries between each realm to become thin, resulting in various physical and hyper dimensional anomalies occurring at random. These phenomena include shifts in gravity, spatial extrusions, and the fabric of reality possibly tearing apart. Perhaps most notably, invisible wormholes that allow matter to move between realms are seemingly born and evaporate at random. As the Convergence reaches its apex, several larger, visible wormholes open in the sky above central locations in each realm, with each portal acting as a window through which parts of other realms can be seen."
>
> http://marvelcinematicuniverse.wikia.com/wiki/Convergence

Smut fungi are a large, diverse, and non-monophyletic group of plant pathogens (Vànky, 2002; Bakkeren et al., 2008; Toh and Perlin, 2016; Agarwal, 2017). Among this diverse group, *Ustilago maydis*, the causative agent of galls on maize, has emerged as a pivotal model for basic scientific research in a variety of areas, including genetic recombination mechanisms, fungal development and cell differentiation, and analyses of host/pathogen interactions, especially those involving effectors. This dominance has been possible due, in part, to a small but cohesive research community and to the number of molecular genetic tools developed, the number of citations of high-profile publications on this organism (Kronstad and Leong, 1989; Schulz et al., 1990; Gillissen et al., 1992; Kämper et al., 1995; Banuett, 1995; Kämper et al., 2006; Lanver et al., 2017; Matei et al., 2018), and the prominence of work describing this fungus at scientific meetings. However, other smut fungi provide the opportunity to explore aspects of fungal biology that differ and/or complement those already characterized for *U. maydis* and its closer relatives, including, *Sporisorium reilianum* (Ghareeb et al., 2011; Ghareeb et al., 2015). While there have been, over the years, scientific gatherings for the broader groups of smut fungi (e.g., XVth Biennial Workshop on the Smut Fungi, Prague, June 11–14, 2006), typically in the last 16 years, the *Ustilago maydis* meetings have been the most consistently convened and attended. Nevertheless, even these international gatherings have declined in recent years. This article describes the efforts of the *Ustilago* community to expand the discussion and exploration to include other smut fungi and to encourage participation by the next generation of young investigators.

* Corresponding author.
E-mail address: anthersmut@louisville.edu (M.H. Perlin).

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2. Goals of the 1st International Ustilago/Smut Convergence at Asilomar

2.1. Scientific foci and training

In attempts to expand the pre-meeting '*Ustilago maydis*' workshops at recent Fungal Genetics meetings in Asilomar (29th and 30th Fungal Genetics Conferences, Pacific Grove, California, March 14–19, 2017 and March 12–17, 2019), scientists investigating other smut fungi were invited. Moreover, in the interest of encouraging the next generation of scientists, a major goal of the workshops was to emphasize the work of young investigators, especially students and post-doctoral researchers, by providing opportunities to showcase their research. The scientific focus of the workshops became an expansion of smut fungal model systems with a focus on genetics related functions and pathogenesis.

2.2. Scientific program

While there was strong representation at the Inaugural and second meetings by investigators of *U. maydis*, less well-known species were also included which enabled us to expand participation and to expose researchers to new fungal systems. Work was presented at the first workshop on *U. maydis*, *Sporosorium reilianum*, *Microbotryum violaceum*, *U. esculenta,* and *Thecaphora thlaspeos*, while phylogenetic knowledge of the poorly understood Ustilaginomycotina was expanded by genomic comparisons among species whose life cycles range from non-pathogens, through animal associated, to plant pathogens. For the Inaugural meeting, after a welcome and introductory remarks that put the meeting in context by Barry Saville (Trent University, Canada), Jan Schirawski (RWTH Aachen University), gave a general overview of the maize pathogens, *Ustilago maydis* and *Sporisorium reilianum*. The vast majority of remaining talks at the Convergence were given by doctoral students. Only those given by post-docs or faculty will be so indicated.

2.2.1. Cell biology of U. maydis

Michael Feldbrügge (Faculty, Heinrich Heine University, Dusseldorf) moderated a short session on cell biology of *Ustilago maydis* related to the infection process. The first of these was from post-doc Sabrina Zander (Heinrich Heine University, Dusseldorf), who discussed the roles of septins during hyphal growth of the fungus. Septins are cytoskeletal GTP-binding proteins with functions in cell polarity, membrane remodeling, cytokinesis and cell morphology. Although the heterooligomeric structure of septins and their subcellular localization had been previously characterized, a precise mechanism of their subcellular assembly and their intracellular transport were unknown. Septins encoded by *cdc3*, *cdc10*, *cdc11* and *cdc12* were required for efficient unipolar growth, indicating their common function during hyphal growth. GFP fusion proteins located the septins to cytoplasmic rings, at septa, in filaments and on moving early endosomes. FRET analysis showed that Cdc3 and Cdc12 interact directly *in vivo*. RNA live imaging of all four mRNAs showed that transport was dependent on the RNA-binding protein Rrm4. FRAP experiments demonstrated that recovery of Cdc3-mCherryN and Cdc12-GFPN occurs simultaneously at distinct subcellular sites and that the rate of recovery was facilitated by the endosomal RNA transport. A model was proposed where local translation promotes the assembly of newly synthesized septins in heteromeric structures on the surface of endosomes. This is important for the long-distance transport of septins and the efficient formation of the septin cytoskeleton.

The next speaker was José "Nacho" Ibeas, (Faculty, Universidad Pablo de Olavide, Sevilla) who described a method to interrogate glycosylation patterns in *U. maydis* proteins. This study was initiated since mutations in essential genes for O-glycosylation (*pmt4*) and N-glycosylation (*gls1* and *gas2*) compromise *U. maydis* virulence on maize, affecting different steps in the infection process (Fernandez-Alvarez et al., 2013). Nacho's group performed proteome analyses, on strains in which the virulence program was activated by Biz1 over-expression, to identify cytoplasmic, secreted and cell wall glycoproteins. Mass spectrometry and MASCOT analysis identified 48 glycoproteins (33 cytoplasmic, 11 secreted and 4 from the cell wall), all dependent of the virulence program activation. A systematic characterization of the role of all these glycoproteins in the infection process is currently in progress.

2.2.2. Regulation of gene expression and genetic programs

This session was moderated by Björn Sandrock (Faculty, University of Marburg). It began with an examination of possible connections, in haploid *U. maydis* cells, between the program that senses nitrogen availability and the mating program. R. Margaret Wallen (University of Louisville) analyzed the roles of different environmental cues that can induce filamentous growth of haploid yeast-like sporidia. In haploid wild type strains of *U. maydis*, low nitrogen availability results in a non-infectious filamentous growth response. Mating between haploid cells is governed by the coordinated expression of genes from the *a* and *b* mating loci in *U. maydis*. Interestingly, the *b* locus must play an additional role in haploid cells, as deletion of the locus prevents filamentation due to nitrogen limitation. Previous work found that the *U. maydis* genome encodes two ammonium transporters, Ump1 and Ump2. While deletion of *ump1* does not yield an observable phenotype, deletion of *ump2* resulted in a loss of low nitrogen induced filamentous growth similar to that observed following *b* locus deletion. Overexpression of the high affinity ammonium transporter, Ump2, resulted in filamentation under non-inducing conditions and rescued the filamentation defect of the *b* locus deletion mutant. In light of this discovery, Margaret and her co-workers hypothesized that Ump2, like its homolog in *Saccharomyces cerevisiae*, acts as a transceptor, both transporting ammonium across the membrane and signaling low ammonium availability; furthermore, the evidence presented showed that functional Ump2 is required for transcriptional regulation related to mating, and that there is interaction at the transcriptional level between *ump2* and the *b* locus.

Niko Pinter (Georg-August-University, Göttingen), discussed the unfolded protein response (UPR) in *U. maydis* and its dependence on Clp1, an important developmental regulator and the decisive factor for the induction of *in planta* fungal proliferation. The UPR is a conserved eukaryotic signaling pathway to counteract endoplasmic reticulum (ER) stress through extensive restructuring of the secretory pathway. UPR is inactive during saprophytic growth and activated after successful penetration of the host. Niko found that unconventional splicing of *cib1* (Clp1 interacting bZIP1) mRNA was a trigger for UPR activation and that the resulting Cib1 protein physically interacted with Clp1. Both genes are crucial for pathogenic development and deletion strains are blocked in development after penetration of the leaf surface. Cib1-Clp1 complex formation results in stabilization of both Clp1 and Cib1 and alters the transcriptional program of the UPR, rendering cells hyper-resistant to ER-stress. Since a constitutively active UPR is deleterious for cell growth, they hypothesized that Clp1-mediated modulation of the UPR prevents deleterious UPR hyperactivation and facilitates a long-term UPR during *in planta* growth. Transcriptional profiling of Clp1-mediated UPR modulation and subsequent gene deletion analysis identified a previously uncharacterized UPR target gene specifically required for biotrophic growth of *U. maydis*. While growth under axenic conditions and ER-stress resistance were not affected, deletion strains were fully avirulent in infection assays. At the 2019 Convergence additional work from this lab found that UPR activity negatively affects phosphorylation of the central MAPK Kpp2, an effect dependent on the dual-specific phosphatase Rok1. These findings indicate a connection between induction of proliferation *in planta* and modulation of the UPR pathway, illustrating UPR's dominant position in a multilayered interaction network that reshapes the secretory pathway and aligns fungal development with the cellular physiology.

In an exciting final presentation on regulation, "The master is switched off, long live the new master: phase specific hierarchical transcriptional control in *Ustilago maydis*," Jonas Ulrich (Karlsruhe Institut für Technologie) outlined a method in which he used a modified Crispr/Cas system to target specific *U. maydis* genomic regions for chromatin-immunoprecipitation. He provided further details on the method during the "business meeting" session at the end of the Convergence. With his method, the targeted DNA is enriched nearly 1000-fold, enabling co-purification of proteins and bound DNA. His experiments recovered Rbf1 protein bound to the promoter of the *dik6* gene. They also revealed that the bE/bW heterodimeric transcription factor triggers the expression of Rbf1 which is the master regulator for most of the biotrophic growth genes whose control was previously attributed bE/bW. While bE/bW expression alone is sufficient to trigger pathogenic development, ectopic Rbf1 expression at least initiates pathogenic development, stressing the central role of both factors. During plant penetration, the function of both proteins was inhibited by interaction with Clp1 and, at later stages, expression of Rbf1 was not detectable. Through microarray-data and ChipSeq analyses, Jonas showed the complex combinatorial control of genes by bE/bW, Rbf1 and Clp1 during the switch to pathogenic development. Both promoter-binding as well as expression data show that at later stages, the transcription factors Biz1 (discussed previously by Nacho) and Hdp2, both essential for plant infection, overtake the central function of Rbf1. These data support a model in which *U. maydis* uses distinct sets of regulators to integrate and/or differentiate the signals received before and after infecting its plant host.

2.2.3. Effectors

The contributions of smut research to our understanding of fungal effectors and their role in plant pathogenesis was addressed in a session moderated by Kai Heimel (Faculty, Georg-August-University, Göttingen) and Regine Kahmann (Director, Max Planck Institute for Terrestrial Microbiology, Marburg). In this session putative bioinformatic identification and functional characterization of effectors were presented. The majority of talks focused on *U. maydis*; however, effector investigation in the head smut of maize, *S. reilianum*, and the anther smut, *Microboryum lychnidis-dioicae* were also highlighted.

The first speaker, Xiaowei Han (MPI for Terrestrial Microbiology, Marburg), presented data indicating that *U. maydis* secretes nearly 300 effector proteins, to manipulate the host and suppress plant defense responses, enabling it to successfully colonize maize. The effector proteins are either secreted to the intercellular spaces (apoplast) or translocated into plant cells to exert their functions. Cmu1, a secreted chorismate mutase, is a translocated effector. It enters plant cells by a currently unknown mechanism and interferes with salicylic acid biosynthesis to promote fungal infection. To gain more insight into its biological function, the structure of Cmu1 was solved by X-ray crystallography of the protein expressed in *E. coli*. The structure revealed a surface-exposed acidic patch, a conserved disulfide bridge and a long loop region situated adjacent to the catalytic site. The relevance of these features was examined in regards to uptake by plant cells, protein stability and allosteric regulation/catalytic activity of Cmu1. In addition, a secreted chorismate mutase from *Sclerotinia sclerotiorum* possessing a chloroplast transit peptide was shown to complement the virulence phenotype of the *cmu1* mutant. Finally, Co-IP followed by mass spectrometry identified a secreted maize protein as a possible target of Cmu1 in the apoplast. Of interest, Xiaowei recently reported further progress in characterizing a maize protein, ZmKWL1, one of the 20 maize-encoded kiwellins, that specifically blocks the catalytic activity of Cmu1 (Han et al., 2019). This is an excellent example of the capability of *U. maydis* research to dissect interactions between fungal and plant proteins during pathogenesis.

Philipp Erchinger (MPI for Terrestrial Microbiology, Marburg) extended this theme by uncovering the interaction of *U. maydis* effector, Ten1, with a maize protein, phosphatase 2C. Ten1 was identified in complementation screens of strains containing deletions of cluster 10A which includes 10 effector-encoding genes. Such deletion mutants display strongly reduced virulence after seedling infection. By generating sub-deletions and by complementing the full deletion of cluster 10A with individual genes, *um03744* (*ten1*) was shown to be a major contributor to virulence. After overexpression in *U. maydis* hyphae, secreted Ten1 protein was detected in axenic culture supernatant. Through a yeast 2-hybrid screen, ZmPP26, a maize protein phosphatase 2C was identified as an interaction partner of Ten1. Interaction was further supported by Co-IP experiments after co-expression of Ten1 and ZmPP26 in *Nicotiana benthamiana*. Moreover, ZmPP26 was detected by mass spectrometry after IP of 3xHA-Ten1 from infected maize tissue 52 h post inoculation. Heterologously expressed ZmPP26 showed strong type 2C-specific phosphatase activity *in vitro*. Ongoing work focuses on the recombinant production of Ten1, testing how the interaction alters phosphatase activity of ZmPP26 and testing a non-interacting allele of Ten1 in plant infections.

Post-doc Lay-Sun Ma (at the time, MPI for Terrestrial Microbiology, Marburg, and currently faculty Assistant Research Fellow, Academia Sinica, Taiwan) completed discussion of *U. maydis* effectors by showing how the secreted Rsp3, which contains several repetitive and Cys-rich domains, protects *U. maydis* hyphae from an anti-fungal maize protein. Functional characterization involved *rsp3* alleles obtained from field isolates that have full biological activity despite length polymorphisms due to reduced or expanded numbers of certain repeats. *rsp3* is highly expressed during the biotrophic stage and is required for virulence and anthocyanin accumulation. Rsp3-HA can be easily detected in culture supernatants when expressed from a constitutive promoter, but displays highly anomalous migration behavior on SDS-PAGE. During biotrophic growth, Rsp3 decorates the surface of biotrophic hyphae. Using co-immunoprecipitation and mass spectrometry, Rsp3 was found to interact with secreted maize DUF26 domain-family proteins. One of these (designated AFP1) was expressed and purified from *N. benthamiana*. AFP1 was able to bind mannose and showed antifungal activity against the *U. maydis rsp3* deletion mutant but not against a strain over-expressing *rsp3*. This suggests that the Rsp3 effector blocks the antifungal activity of the DUF26-containing maize protein, presumably by inhibiting its binding to the fungal cell wall. The research is an example of the capabilities for functional analyses in the *U. maydis/Z. mays* pathosystem.

The presentation of data from other smuts began with Nisha Agrawal (RWTH Aachen University), identifying an interaction between *S. reilianum* effector Sad1 and maize protein ZmRGLG1 which leads to the suppression of apical dominance in maize ears. When expressed in *A. thaliana*, GFP-Sad1 localizes to the cytoplasm and the nucleus and leads to increased inflorescence branching. From a yeast 2-hybrid screen, the maize E3 ubiquitin ligase ZmRGLG1 was identified as one of the strongest interactors of Sad1. Bimolecular fluorescence complementation (BiFC) showed that Sad1 interacts with ZmRGLG1 at the plasma membrane in *N. benthamiana*. ZmRGLG1 has a proposed myristoylation site at its N-terminus. Accordingly, ZmRGLG1-GFP localized to the plasma membrane when expressed in *N. benthamiana*, but not when the N-terminal Gly was mutated to Ala. AtRGLG1 and AtRGLG2 are the homologs of ZmRGLG1 in *A. thaliana.* In *A. thaliana*, AtRGLG1/2 is known to move from the plasma membrane to the nucleus upon stress, and there to function in dampening the stress response. Nisha hypothesized that interaction of Sad1 with ZmRGLG1 interferes with ZmRGLG1 function or transport into the nucleus, which would lead to a prolonged stress response. A prolonged stress response has been suggested to cause suppression of apical dominance, suggesting a mechanism whereby Sad1 might lead to suppression of apical dominance by its inhibition of ZmRGLG1.

The last oral presentation sessions in the Convergence highlighted expanding molecular genetic investigations with other smut fungi. Dominik Begerow (faculty, Ruhr-Universität Bochum) and post-doc Martin Kemler (Ruhr-Universität Bochum) introduced the anther smut systems exemplified by the *Microbotryum violaceum*. This fungus is part of a fungal species complex whose members infect over 100 host plant species and provide a fascinating model in which to examine molecular mechanisms of host specificity. Moreover, the evidence of host shifts for particular *Microbotryum* species makes these systems a potential avenue for investigating the evolution of emerging infectious diseases. The relatively recent addition of comparative genomics (Perlin et al., 2015; Badouin et al., 2015, 2017), transcriptomics (Perlin et al., 2015; Toh et al., 2017a, 2017b), and *Agrobacterium*-mediated Transformation (Toh et al., 2016), has made significant advances in understanding the role of effectors in members of this complex. V. Swathi Kuppireddy (University of Louisville) described the potential host targets identified for effectors of one member of the complex, *M. lychnidis-dioicae*. Her group characterized the roles of several effectors by identifying their host targets using yeast 2-hybrid analysis. Some interesting potential plant protein interactors were identified, including at least one predicted to be involved in pollen dehiscence and pollen tube elongation. Further experiments will reveal the significance of these fungal effectors in pathogenicity and their possible location inside the host plant during infection. One avenue for these investigations was presented at the second Convergence in 2019, where additional insights were gained by expressing a *M. lychnidis-dioicae* effector in *A. thaliana* and investigating its localization in the absence of the fungus. Overall, this study will provide greater understanding of the mode of pathogenicity of *Microbotryum* species.

Post-doc Alex Harkess (Donald Danforth Plant Science Center) followed up with a discussion of his efforts to examine the host side of the interaction between *M. lychnidis-dioicae* and *Silene latifolia*. In particular, Alex focused on efforts to understand *Microbotryum*-mediated sex conversion of *Silene latifolia.* It has long been observed that *M. lychnidis-dioicae* was able to infect *S. latifolia* female flowers and lead to sexual conversions. This peculiar fungal parasitism can cause *S. latifolia* XX females to produce rudimentary anthers inside which the fungus propagates, but interestingly the infection of XY male plants does not produce female organs. This parasitism could be a key to identifying the master sex determination genes on the *Silene* XY sex chromosome pair. To understand the molecular mechanisms of this sexual transition, Alex and his collaborators are sequencing mRNA, small RNA, and PARE ("degradome") libraries from pre- and post-infection *S. latifolia* flowers, as well as attempting genetic transformation of both the host species and its fungal parasite for Cas9-based genome editing. Given that there may exist species co-diversification of fungus and host, they hypothesize that the transition from hermaphroditism (self-compatibility) to dioecy (outcrossing) in *S. latifolia* may have been spurred by a plant-fungus evolutionary "arms race".

Two additional talks featured, for many of us, lesser-known smut systems of potentially great interest. Wei-Chiang Shen (Faculty, National Taiwan University) detailed work with *Ustilago esculenta* Henning, a smut fungus associated with a wild rice species, *Zizania latifolia* Turcz. This fungus is responsible for the formation of a prized vegetable in East Asia, water bamboo or makomotake, which is swollen galls at the base of the plant resulting from infection by the smut fungus. Sexual differentiation of the fungus is required for the establishment of its association with the plant and the development of edible galls is stimulated by an alteration of the phytohormone equilibrium. In this study, Wei-Chang presented a characterization of the mating type loci of *U. esculenta* and determined its genetic diversity. *U. esculenta* isolates were obtained from teliospores and gall tissues of field materials collected in Taiwan and Japan. Using NGS and PCR screening approaches they discovered conservation of a pheromone/pheromone receptor system as well as homeodomain transcription factors and identified three idiomorphs. Genetic evidence indicates that *U. esculenta* has a bipolar heterothallic mating system and extremely large mating type locus. Transposon insertion at the mating type locus and simple sequence repeats were used to develop molecular markers for fingerprinting *U. esculenta*. This enabled Wei-Chang to uncover genetic diversity and create a screen for breeding new varieties of *U. esculenta* for use in commercial water bamboo production.

Finally, Kristin Boesch, (Heinrich-Heine-University), revealed a convenient system to study the host side of smut interactions. Genetic manipulation of crop host plants is still difficult because of their complex genomes. For this reason, Kirsten characterized the interaction of the smut fungus, *Thecaphora thlaspeos,* with the model plant, *Arabidopsis thaliana*. *T. thlaspeos* infects different Brassicaceae throughout Europe, among them the perennial plant, *Arabis alpina*. Its lifecycle differs in two important steps from crop smut fungi such as *U. maydis*: First, teliospores only germinate in the presence of a plant-derived signal. Second, there is no dimorphism. Filaments emerge from teliospores that are directly infectious. Importantly, these filaments can be propagated as haploid cultures enabling genome sequencing and genetic manipulation. Of interest, the genome encodes components of RNAi machinery, a system absent in *U. maydis* but present in *S. reilianum* and *U. hordei*. Cross-kingdom RNAi is used by *Botrytis cinerea* to modulate the immune system of *A. thaliana*. Based on reduced virulence of the DICER deletion mutant, it is hypothesized that siRNAs are generated in the fungus, transported into the cells of the host plant and there, silence expression of specific genes involved in immunity. This new model system can be used to explore how *T. thlaspeos* uses RNAi and ultimately to investigate whether this smut fungus also utilizes cross-kingdom RNAi during infection.

2.2.4. Phylogenetic analyses of host preference

Martin Kemler began the concluding portion of the Convergence with an evaluation of the phylogenetic relationships among the smut fungi. He presented an examination of the evolution of host preference and sorus location in the plant parasitic genus *Microbotryum*. Species in this genus have radiated, with the host species, following establishment of infection in individual species of different monophyletic genera within the Caryophyllaceae. However, the *Microbotryum* host range extends beyond the Caryophyllaceae to other plant families. Additionally, there has been an evolution in plant colonization among the *Microbotryum* species with sorus formation occurring in locations other than the anthers. Here, Martin presented an updated phylogenetic approach using a denser sampling of species from different host families. The work explored the following questions: (1) What is the ancestral state of the host genus parasitized by the genus *Microbotryum*? (2) What is the ancestral state of sorus location in the genus *Microbotryum*? (3) Are monophyletic clades of parasites found on monophyletic groups of hosts? The results corroborated previous findings that the ancestor of the genus evolved on plants in the Polygonaceae where it most likely formed it spores in inflorescences. As in the Caryophyllaceae, many monophyletic groups of hosts have been colonized once with subsequent radiation. These results were discussed in light of some life-history traits of both hosts and parasites.

Stephen Mondo (Faculty, Fungal Genomics, Joint Genome Institute) next explored genomics and sampling of the Ustilaginomycotina subphylum. He pointed out that despite their importance as plant and animal pathogens, as a whole the Ustilaginomycotina remain one of the most poorly understood branches of the Basidiomycota. As genomes are becoming available for more orders, comparative genomics is becoming an attractive tool for further characterization of this subphylum. At the JGI, they had annotated genomes available for 16 Ustilaginomycotina, representing 9 different orders. Most of these genomes are small (ranging from 8 to 27 Mb) and very compact. However, this sampling still represents a small subset of the total diversity present within Ustilaginomycotina. Stephen discussed the sequencing progress and preliminary results comparing these genomes to one another and other fungi. Through collaborations with the Ustilaginomycotina community, the aim is to provide a comprehensive set of genomes to facilitate deeper exploration of this subphylum.

The Convergence oral presentations ended with a lively discussion, initiated by Dominik Begerow, on the virtues of re-thinking the phylogeny of the genus *Ustilago*, especially whether to redefine the type species based on *U. maydis* vs. *U. hordei*. The discussion, politely heated at times, actually began online prior to the meeting, so a number of those in attendance were prepared with cogent reasoning for their respective positions. Despite proposals to re-think the genus as *Mycosarcoma* (McTaggart et al., 2016), the consensus of those in attendance and online was to conserve *Ustilago*, with *Ustilago maydis* as its type and most important species. This being the overwhelming consensus at the meeting, i.e., to conserve the name *Ustilago* (*Basidiomycota*) with the current type, a signed letter was delivered to the International Association for Plant Taxonomy Committee for Fungi of IAPT, asking for a moratorium not to change the type of *Ustilago* at least for the next six years until the next botanical congress.

2.2.5. Additional highlights from the poster session

In addition to the oral presentations, a poster session enabled further areas for discussion. We highlight here just a few of the posters that provided material not discussed directly in the oral sessions. Nicole Ludwig (MPI Terrestrial Microbiology, Marburg) added to the emphasis on effectors at the meeting. Systematic deletion of the most highly expressed effector genes early in infection resulted in the discovery of three mutants unable to cause disease. Their mutant phenotype resembled previously identified *stp1* (stop after penetration) mutants, and the newly identified genes were designated *stp2*, *stp3* and *stp4*. A similar phenotype was also observed for mutants lacking the essential effector *pep1* (Döhlemann et al., 2009).

Barry Saville (Trent University, Canada) presented the results of > 1 billion paired-end RNA-sequence reads obtained from haploid cell, dikaryon and teliospore RNA of *U. maydis*, haploid cell RNA of *U. hordei*, as well as haploid and dikaryon cell RNA of *S. reilianum*. His group used Trinity for transfrag assembly, and PASA to create updated gene models which were categorized with Cufflinks Cuffcompare. They used reverse transcription PCR to assess and confirm representative genes that were predicted for the first time by these analyses as well as genes with novel annotation features. They concluded that: (1) the number of transcriptional units in smut genomes is twice the number of annotated protein-coding genes, (2) the vast majority of the identified intergenic and antisense transcripts did not contain ORFs, (3) there was antisense transcript conservation among the smut fungi, and (4) the enrichment of functional categories among genes with the conserved presence of antisense RNAs suggested a genome-wide, non-RNAi-mediated, antisense RNA influence on gene expression in smut fungi. These results were subsequently published (Donaldson et al., 2017).

Moreover, the poster sessions provided an avenue for a contingent of MS and doctoral students from the lab of Jose Ruiz-Herrerra at CINVESTAV (Irapuato, Mexico) to highlight several areas of investigation with *U. maydis*. Domingo Martínez-Soto et al. described a transcription factor, Ztf1, differentially regulated in the experimental host, *A. thaliana*, as well as in the natural host, *U. maydis*. They found that Ztf1 was required for appropriate yeast-to-mycelium transition in response to fatty acids, as well as for full virulence in both host systems. Fernando Pérez-Rodríguez examined the role of polyamines in the mating process, utilizing *odc*$^-$ (ornithine decarboxylase) polyamine auxotrophic strains of compatible mating types; at low polyamine levels, mating was severely reduced, as was expression of genes involved in the mating pathway, particularly *mfa1* and *mfa2* genes encoding pheromone precursors. José Alejandro Sánchez-Arreguin et al. presented further observations on fruiting body (basidiocarp) development of *U. maydis* and the role of homologues of White Collar (WC) photoreceptors. Mutants in each one of the three WC homologue genes, *WC1a, WC1b*, and *WC2*, were produced and analyzed. The *wc1b* and *wc2* mutants were severely affected in basidiocarp formation when illuminated with white, blue or red light, whereas *wc1a* mutants formed basidiocarps in all light conditions. These results suggested that Wc1b acts as a photoreceptor or/and that Wc1a and Wc2 are involved in a photoreaction dependent on a phytochrome. Wc1b was also involved in virulence to maize. Cinthia V. Soberanes-Gutiérrez conducted experiments to determine whether the *U. maydis pep4* gene, that encodes the acid vacuolar proteinase A, ortholog to human Cathepsin D, was important for autophagy in *U. maydis*. Her observations showed the accumulation of autophagic bodies within the vacuole of *Δpep4* cells, in contrast to their lack of accumulation into vacuoles of wild-type cells, thus demonstrating that the proteinase A deficient mutant is affected in its capacity to lyse the autophagic bodies, and accumulated cytosolic material in the autophagosomes present within the vacuole.

The second workshop (2019) built on the foundations established in the Inaugural meeting (Sections 2.2.1–2.2.5), with some researchers updating ongoing projects and others expanding knowledge on molecular aspects of fungal development during *in planta* growth. This included presentations on nitrite metabolism and lipid signaling, cell biology, transcription factors, transcriptome analysis of developmental stages and hybrid dikaryons, and the impact of secreted proteins including ribonucleases and effectors. The topic list was rounded out by presentations on population structure, hybridization, and using *U. maydis* as a production platform for bioactive compounds. A goal of the second workshop was nicely illustrated by several talks outlining the expansion of effector research beyond *U. maydis* to *S. reilianum* and *M. violaceum*.

2.3. Professional development

These meetings also sought to expand the diversity of participation, both in terms of attendance and the range of scientific topics covered. In terms of ethnicity, efforts were explicitly made in an attempt to increase attendance and participation by young investigators from Mexico, where a reasonably large group of scientists has been working with *Ustilago maydis* for over 25 years (Examples of research include: Guzman and Sanchez, 1994; Xoconostle-Cazares et al., 1996, 1997; Ruiz-Herrerra et al., 1996; Reyna -Lopez et al., 1997; Ruiz-Herrerra and Martinez-Espinoza, 1998; Ruiz-Herrera et al., 1999; Juarez et al., 2011; Bautista-España et al., 2014; Martínez-Soto et al., 2015; Cervantes-Montelongo et al., 2016; Leon-Ramirez at al., 2017; Olicon-Hernandez et al., 2019; Cervantes-Montelongo and Ruiz-Herrera, 2019; Sánchez-Alonso et al., 1996; Sánchez-Alonso and Guzmán, 1998; Schuster et al., 2017; Valverde et al., 1995). In addition, we sought participants from labs in Spain and Latino students from the US. In order to support the attendance of students and post-doctoral researchers from this demographic, a Meetings grant was obtained from the US National Science Foundation that provided, among other things, financial support to defray travel costs to the meeting and registration. In addition, the grant was used to recognize outstanding student participants with a monetary award.

We encouraged students and post-doctoral researchers to present their "just-in-time," as-yet-unpublished data, in a safe environment, with the understanding of those attending the meeting that this early access was a privilege not to be taken advantage of.

The one-day workshops began with evening social gatherings, at which participants shared refreshments, including delicacies and specialties from their respective "home towns." This provided an opportunity to break the ice, before the scientific presentations on the day of the workshop. During the first Convergence, oral presentations were provided by nine doctoral students and four post-docs, in a program that introduced and gave an overview of the varied systems under study. In addition, poster presentations (16 from doctoral students and nine from post-docs) were available for discussions during breaks for refreshments and lunch. In the second workshop the posters were up during the social gathering for discussion and the workshop day included oral presentations from eleven graduate students and three post docs. The second workshop ended with a tribute to Professor Doctor Regine Kahmann. In honor of her transition from Director and Head of the Department of Organismic Interactions at the Max Planck Institute for Terrestrial Microbiology (for 19 years) to Interim Director, several perspectives of her career were presented by her collaborators/

colleagues. Michael Bölker described their work together unraveling the mysteries of Mu phage transposition and the site-specific recombination of the G-loop associated with host switching. Jörg Kämper reminisced about their years together at the Institute for Genebiological Research in Berlin and Regine's penchant for discussing science with students and post-docs, preferably at a Bier Garten or Hofbräuhaus. The tribute concluded with personal memories shared by Flora Banuett of the beginnings of the work on *Ustilago maydis,* bringing together Regine with Flora and Ira Herskowitz. In the end, all made merry on toasts with Kentucky 1792 Bourbon and top-shelf Canadian Rye.

3. Prospective measurements of outcomes since the meetings

An important goal of the meetings was to provide mentoring opportunities for graduate student and post-docs. Our time together at lunch and meetings during the fungal genetics conference following the Ustilago/smut Convergence enabled this. We also have endeavored to track the progress and transitions for the student and post-doc participants in the first meeting. As of the preparation of this review, 2 MS students have continued on into PhD programs; 3 PhD students have begun Post-doctoral positions, while one additional Post-doc moved on to a new Post-doc position. Moreover, 1 Post-doctoral researcher has since made the transition to Faculty, where she will continue work with *Ustilago maydis* in her own lab. We intend to continue to track such progress, as well as publications for participants (see below) for the next several years. It is notable that only one student from the 2017 workshop also attended the 2019 which means that nine new students presented at the second meeting.

Students and post-docs who attended the first meeting have, since, published work often including that presented at the workshop. The publications from these individuals include: Kuppireddy et al., 2017; Wallen and Perlin, 2018; Paul et al., 2018; Zander et al., 2018; Aschenbroich et al., 2018; Han et al., 2019; Lanver et al., 2017; Ma et al., 2018a, 2018b; Martínez Soto et al., 2015, 2018; Martínez Soto and Ruiz-Herrera 2016, 2017; León-Ramírez et al 2017; Soberanes-Gutierrez 2015; Juárez-Montiel et al., 2018; Soberanes-Gutiérrez et al., 2019; Sánchez-Arreguin et al., 2017; and Schuler et al., 2018. A. Harkess also visited the first convergence as a Post Doc from the Danforth Center, to present on fungal manipulation of host plants; his many publications since then are plant focused and are not included here.

The convergence also provided a forum in which collaborations were established and material exchanges were set up to facilitate future research.

4. Future goals

We plan to continue to follow the progress of students and post-doctoral participants of the two Ustilago/Smut Convergence workshops for 3–5 years. We will convene the meetings every two years, in conjunction with the Fungal Genetics Meetings or similar meetings and make changes, as appropriate, based on exit surveys of the meeting. Participation in the 1st Convergence by Latino students was facilitated through NSF Meetings support. In contrast, for the 2nd Convergence, there was no participation by those from Spanish-speaking countries or backgrounds, suggesting strongly that financial considerations likely prevented these scientists from joining their colleagues at the meeting. We will, therefore, seek additional funding to bring in more students/post-docs who would otherwise be unable to attend due to financial constraints. An exit survey of the most recent meeting in fact identified cost as a major impediment to attendance for students and post-docs. In the same survey, 100% of respondents either strongly agreed or agreed with the statement: "I liked that this meeting focused on presentation opportunities for students and post-docs."

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