Maize Tumors Caused by *Ustilago maydis* Require Organ-Specific Genes in Host and Pathogen

David S. Skibbe,1* Gunther Doehlemann,2* John Fernandes,1 Virginia Walbot1†

Infection of maize by corn smut (*Ustilago maydis*) provides an agronomically important model of biotrophic host-pathogen interactions. After penetration of the maize epidermis, fungal colonization of host tissue induces tumor formation on all aerial maize organs. We hypothesized that transformation of different primordia into plant tumors would require organ-specific gene expression by both host and pathogen and documented these differences by transcriptome profiling. Phenotypic screening of *U. maydis* mutants deleted for genes encoding secreted proteins and maize mutants with organ-specific defects confirmed organ-restricted tumorigenesis. This is the foundation for exploring how individual pathogen effectors, deployed in an organ-specific pattern, interact with host factors to reprogram normal ontogeny into a tumor pathway.

*Ustilago maydis*, the causal agent of corn smut disease, is a basidiomycete fungus parasitizing only maize and its wild progenitor teosinte (*Zea mays L.*). (*U. maydis*) elicits large tumors on all aerial organs, where it completes pathogenic development by forming teliospores, its predominant dispersal mechanism. While tapping the nutritional supply of colonized cells, *U. maydis* establishes an intimate interaction with living hosts (*Fig. 1, A and B*) by suppressing plant defenses while tapping the nutritional supply of colonized cells. This interaction is maintained by secretion of fungal effector proteins, which either act at the biotrophic interface between pathogen and plant cell or are translocated into the host cytoplasm (*Fig. 2*). Sequencing of the *U. maydis* genome and transcriptome profiling during seedling infection identified 12 gene clusters encoding primarily uncharacterized, predicted secreted proteins expressed in planta (*Fig. 3*). Infection assays with maize seedlings infected five of these clusters as functionally involved in tumor formation (*Fig. 4*).

Extensive analysis of bacterial and oomycete effector proteins has identified several mechanisms for host cell manipulation (*Fig. 5*); however, to date there is no evidence that the action or expression of any pathogen effector is tailored to individual host tissues. This is surprising because *U. maydis* is tumorigenic in leaves, stems, and flowers, and these organs and constituent maize tissues and cell types express distinctive developmental genes (*Fig. 6*), as is true in any complex eukaryote. Furthermore, maize mutations that disrupt normal development can enhance or suppress tumor progression (*Fig. 7*), demonstrating that host developmental status is important in the biotrophic interaction.

To define the genes expressed by maize and *U. maydis* during infections culminating in tumors (*Fig. 1, C and D*), transcriptomes were assessed on a microarray with probes to ~6700 annotated *U. maydis* genes (*Fig. 8*), 4941 of which showed only background levels of hybridization with maize RNA in control hybridizations (*Fig. 9*). During the arms race with the multilayered plant defense system, plant pathogens such as

1. Department of Biology, Stanford University, Stanford, CA 94305–5020, USA. 2Max Planck Institute for Terrestrial Microbiology, D-35043 Marburg, Germany.
2*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: walbot@stanford.edu

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only 7% of maize transcripts were altered by *U. maydis*, and the up or on (1118) (Fig. 2A) and down-regulated or off (1436) (Fig. 2C) classes were almost equivalent. Fungal infection alters only one-third as many genes in tassels, showing that formation of floral tumors was accompanied by less reprogramming of development compared to leaves (12).

Host responses were primarily organ-specific in both the up- and down-regulated classes. There were only 223 commonly up-regulated (Fig. 2A) and 23 on (Fig. 2B) transcripts. Although many genes were down-regulated or off among organs, only 135 were commonly down-regulated in all infected organs (Fig. 2C). We found differences in expression of defense-related genes in individual organs, e.g., the gene encoding pathogenesis-related protein 10 was strongly induced in seedling leaves but not in adult leaves. Hormone and metabolism genes were also differentially expressed during infection: gibberellin acid-oxidases, auxin transporter *pin1*, and auxin-response *if-3* were up-regulated in adult but not in seedling leaves. These data establish that maize organs display discrete responses to *U. maydis* infection.

*U. maydis* expresses many genes during seedling infections, particularly the class encoding secreted proteins, which are not detected during saprophytic fungal growth (4). Notably, *U. maydis* exhibits expression patterns specific to infection location (Fig. 2D). Nearly one-third (*n*=353) of fungal transcripts were induced in all three organs, with another third (*n*=1412) present in two organs. Almost 1200 fungal genes were uniquely expressed in adult leaves, with smaller numbers in seedling (*n*=296) and tassel (*n*=88). That more than 36% of the fungal transcriptome profile is organ-specific at 3 dpi suggests that successful host colonization requires deployment of gene products that can interact with maize proteins characteristic of three distinct developmental states. The specificity of interaction is also true at 9 dpi, when tumors are evident in adult leaves and tassels: In addition to 915 genes in common, *U. maydis* expresses 223 genes specifically in adult leaves and 714 in tassels (table S1).

There are 554 in silico–predicted secretory proteins encoded by *U. maydis*, collectively designated as the secretome (13); these are of particular interest for biotrophic fungal development. Most of these proteins were *U. maydis*–specific and lacked similarity to known enzymes (13). Of these, 325 were evaluated with high-confidence probes, resulting in the identification of 261 genes that were expressed at least one infected versus mock sample type at 3 dpi (Fig. 2E). Only 21% (*n*=70) of these genes were expressed in all three maize organs at 3 dpi whereas 45% (*n*=118) showed organ-specific expression: 28 in seedling leaves, 86 in adult leaves, and 4 in tassels, a trend that continued at 9 dpi (Fig. 2F).

In a complementary approach, phenotypic screening of plant and fungal mutants tested the necessity of organ-specific host and pathogen signals to make tumors. Maize mutants with defects in hormone signaling were scored for tumor formation in seedlings, adult leaves, and tassels as summarized in Table 1. *Dwarf8* (*D8*), which is disrupted in gibberellin hormone signaling, has drastically reduced shoot size (14). Infected *D8* seedlings support extensive tumor formation but completely lack adult tissue tumors (fig. S1A), indicating that gibberellin signaling is dispensable for tumor formation in seedlings but is indispensable in adult tissues. This observation is also consistent with the transcriptional induction of gibberellin-acid-oxidases only in the adult tissue. Furthermore, the auxin hormone response mutant *sparse inflorescencel* (spil) (15) shows normal vegetative tumors but essentially no floral tumors. The *Knotted1* (*Kn1*) mutant displays excessive adult leaf growth (16) from disrupted gibberellin regulation (17). *Kn1* has normal symptom formation in seedlings but displayed more frequent and larger adult leaf tumors and larger tassel tumors (Table 1 and fig. S1, B and C). Three premeiotic male-sterile mutants all produced normal seedling and adult leaf tumors but lacked floral organ tumors (Table 1). These observations demonstrate organ-specific control of tumor progression in maize growth control mutants.

To address the organ-specific role of *U. maydis* secretome proteins, deletion mutants in the *SG200* solopathogenic strain for 12 gene clusters encoded...
ing 71 secreted proteins (4) were inoculated on adult leaves and tassels of W23 inbred maize. In addition, all 12 mutants were reevaluated in W23 seedlings to confirm previous phenotypes reported on Golden Bantam corn (4). Five of the *U. maydis* mutants showed significantly different virulence depending on the organ infected: Δ5B was nonpathogenic (failed to penetrate beyond one cell) on seedlings (Table 1); however, in adult leaves at 9 dpi, it caused extensive chlorosis spreading around infection sites indicative of successful fungal penetration (Fig. S2C). Δ2A was hypervirulent on seedlings (4) but had a lower frequency and smaller tumors in adult leaves and a normal frequency of larger tumors in tassels (Table 1). Δ10A showed reduced frequency and size of seedling and adult leaf tumors but caused developmental arrest of the tassel, which formed no or only a few tiny tumors. The Δ9A mutant showed wild-type frequency of tumors on seedlings but exhibited reduced virulence on adult leaves and, similar to Δ10A, caused developmental arrest in tumor-free tassels (Table 1 and fig. S2, D and E). Most noteworthy are the findings for the Δ19A mutant deleted for 24 secretome proteins. This mutant did not cause any seedling tumors (4) but induced formation of tumors at a frequency comparable to that of SG200 in adult leaves and tassel, although the tumors were smaller (Table 1 and fig. S2F).

Consistent with these observations, the genes within the secretome clusters showed quantitative expression differences at 3 dpi in each maize organ (Fig. 3 and table S5). We found 39 organ-specific gene expression differences among the 47 secretome proteins contained in the five clusters with organ-specific phenotypes (Fig. 3). In particular, 15 genes of cluster 19A, which is essential for tumor formation in seedlings but dispensable in adult tissue, showed significantly reduced expression in tassel and adult leaves compared with seedling infections at 3 dpi, whereas only two genes showed increased expression in the tassel relative to seedlings (Fig. 3). In contrast, two genes of cluster 9A, which is more important for symptoms in adult tissue than in seedlings, were expressed at similar levels in all three organs.

Collectively, the gene expression and genetic findings demonstrate organ-specific expression of *U. maydis* effectors, showing essential roles in tumorigenesis. These secretome proteins, which likely constitute the majority of effector molecules eliciting host responses, indicate deployment of different “weapons” tuned to host organ properties. Smut fungi typically infect host seedlings and spread systemically in zones of proliferating cells during plant development; however, they cause symptoms exclusively in inflorescences (18). *U. maydis* is unique among smuts in converting leaves and stems into tumors; a larger suite of *U. maydis* genes is involved in tumor formation in vegetative organs than in the tassel. Floral tumors may draw on pathogenic factors that are more highly conserved with other fungi and that could serve general roles during pathogenesis in maize such as the *U. maydis* genes required for fungal penetration of plant cells (19).

Individual maize organs express distinctive proteins, and mutations that alter organ development can enhance or repress tumorigenesis by *U. maydis* (7) (Table 1). Mirroring the role of host differential gene expression is the unexpected transcriptional plasticity of *U. maydis* during infection of seedlings, adult leaves, and tassels and the observation that some deletion mutants alter tumor formation only in specific organs. We conclude that reprogramming by *U. maydis* may involve dedifferentiation from normal maize cell fates into new pathways, utilizing repression and de novo activation of different developmental programs in each infected proliferative zone.

We propose a model with two phases in this pathogenic interaction. First is establishment of compatibility, which most likely depends on universal pathogenicity factors to suppress plant defenses during fungal penetration (12, 20).
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Second, disease progression requires response to maize organ-specific properties so that U. maydis can tailor effector deployment to redirect physiological and developmental processes to a specific organ primordium. Sequential refinement of specificity may be of particular importance in this biotrophic interaction, which lasts 14 days from host penetration to fungal spore release. Within this conceptual framework, the next step is elucidation of distinct fungal and host factors interacting in a tissue-specific and temporal context. This new knowledge will clarify how organ-specific factors modulate biotrophy and, ultimately, tumor formation.

References and Notes
9. Materials and methods and supporting materials are available on Science Online.
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Supporting Online Material
www.sciencemag.org/cgi/content/full/328/5974/89/DC1
Materials and Methods
References
Figs. S1 and S2
Tables S1 to S6
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Cryptic Sex-Ratio Bias Provides Indirect Genetic Benefits Despite Sexual Conflict
Robert M. Cox* and Ryan Calsbeek

When selection favors sexual dimorphism, high-fitness parents often produce low-fitness progeny of the opposite sex. This sexual conflict is thought to overwhelm the genetic benefits of mate choice because preferred males incur a cost through the production of low-fitness daughters. We provide a counterpoint in a lizard (Anolis sagrei) that exhibits sexual conflict over body size. By using mate-choice experiments, we show that female brown anoles produce more sons than daughters via large sires but more daughters than sons via small sires. Measures of progeny fitness in the wild suggest that maximal fitness payoffs can be achieved by shifting offspring production from daughters to sons as sire size increases. These results illustrate how the resolution of sexual conflict can restore the genetic benefits of mate choice.

Because of their divergent reproductive roles, males and females often experience different selection pressures acting on the same phenotypic traits (1). However, sharing a common genome constrains the sexes from evolving independently in response to these antagonistic selection pressures (2–4). This can result in a genomic tug of war referred to as intralocus sexual conflict (5–7). When such conflict is widespread throughout the genome, high-fitness parents may actually produce low-fitness progeny of the opposite sex (8–14). This outcome can override the potential genetic benefits of mate choice because preferred males incur a net fitness cost through the production of low-fitness daughters (8–10). When sire genotypes have differential fitness effects on sons versus daughters, females are predicted to alter progeny sex ratio accordingly (15). We tested whether progeny sex-ratio bias can facilitate the sex-specific inheritance of good genes, thereby preserving the genetic benefits of mate choice in the face of sexual conflict.

The brown anole lizard (Anolis sagrei) exhibits signatures of intralocus sexual conflict over body size (Fig. 1). On average, adult males are 30% longer and 150% heavier than adult females (16). Selection creates the potential for sexual conflict by favoring large size in males and intermediate size in females (17). However, anoles have also evolved several mechanisms that may resolve this conflict. First, body size and other morphological traits are heritable within each sex but exhibit negative genetic correlations between the sexes (18). Second, paternity analyses of wild populations reveal that females produce more sons via large sires but more daughters via small sires (18). This suggests a form of cryptic sex-ratio bias that may allow females to adaptively sort genes with sex-specific fitness effects into sons and daughters.

Fig. 1. Female anoles bias progeny sex ratio as a function of sire body size. Data are least-squares means ± 1 SEM from analyses weighted by the total number of progeny produced by each dam-sire pair. Size is dichotomized relative to the population mean.

Fig. 2. Natural selection on three phenotypic traits differs between male and female progeny. Data are selection differentials ± 1 SEM derived from regressions of relative survival on trait values standardized to the population mean in unit variance. Asterisks indicate statistical significance (P < 0.05) on the basis of logistic regression.