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ON THE NATURE OF THINGS: ESSAYS

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Pushing the boundaries of organ identity: Homology of the grass lemma

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Plant form is astonishingly diverse. The field at the intersection of evolution and development ("evo-devo") aims to reveal how this form arises via changes in development, leveraging comparisons between species across scales of evolutionary time. These comparisons reconstruct the trajectory of evolution and reveal how species adapt and diversify. Flowers, in particular, are often the focus of evo-devo studies. Although flowers are usually constructed from the same component parts, these parts vary in structure and arrangement, and homologies can be unclear.

Despite their small, nonshowy flowers, the grass family is valuable in evo-devo, offering diversity and multiple experimental systems. Grasses are also the dominant monocot system for molecular developmental genetics. Indeed, monocots are often wholly represented by grasses in molecular biology studies. How we recognize homologies of grass floral organs directly affects experimental design and interpretation. Therefore, it is crucial that we are precise in defining grass flower homologies.

MORPHOLOGY AND MOLECULES IN DEFINING HOMOLOGY

Homology is a useful concept in evo-devo for thinking about the origins and relatedness of structures across species. We use homology to refer to a relationship among structures that correspond to one another because of shared descent. In contrast, with homoplasy, similarity is due to convergence (Wake, 2011). Homology is particularly valuable for understanding how evolutionary change affects the same starting material to result in different morphologies.

One effective way to evaluate homology is with Remane's criteria. The three criteria are (1) equivalent organ position, (2) structural similarity, and (3) transitional states or intermediate forms (Remane, 1971). In addition, genetic information has become accessible for many species, and gene networks shared in organ development can provide evidence for homologies (Svensson, 2004). However, many organs may share gene expression, homologous or not. Character identity networks (ChINs) could be used as additional criteria for homology. Character identity networks are putative networks of genes that pattern homologous organs' identities (e.g., leaves, petals, sepals) regardless of character (e.g., shape, size, color). Importantly, gene function and genetic interactions are critical in defining ChINs, not just lists of shared or divergent gene expression patterns (Wagner, 2014). Although they have not yet been defined in plants, ChINs that regulate, for example, animal brain regions have been identified, using *Drosophila* as a study system (Bridi et al., 2020). Together, both morphological criteria and gene networks help make homology assessments.

GRASS LEMMA HOMOLOGY IS UNCLEAR

Grass flowers belong to units called spikelets, subtended by two bracts, called glumes. A grass flower usually consists of a lemma, palea, lodicules, and stamens surrounding a central pistil (Figure 1). The lodicules occur in pairs or sets of three

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and are likely homologous to petals. The palea is external to the lodicules and is often membranous and two-keeled (having two adaxial ridges). The lemma subtends the flower and is typically a sturdy, single-keeled, leaf-like structure, which may bear a bristle-like awn. Organs within the spikelet may be modified, as in *Buchloe dactyloides*; reduced, as in *Anthoxanthum odoratum*; or repressed, as is the palea of *Alopecurus pratensis* or the stamens, carpels, lodicules, and palea of some *Cynosurus cristatus* flowers (Figure 2).

While the homologies of pistils, stamens, and lodicules are clear, there are two main hypotheses for lemma and palea homology. The first, based on morphology and development, places the palea in the outermost floral whorl, and the lemma as a subtending bract (Kellogg, 2000). The second, based on molecular genetics, places the lemma and palea in the outer floral whorl, homologous to sepals (Lombardo and Yoshida, 2015). Here, we review these hypotheses and discuss a third, where the lemma is considered a novel organ, intermediate between a bract and a sepal.

COMPARATIVE MORPHOLOGY SUGGESTS LEMMAS ARE HOMOLOGOUS TO BRACTS

Comparative morphology and leveraging evolutionary history often help resolve homologies. *Streptochaeta angustifolia*, sister to all other grasses, has been studied for this purpose. The pseudospikelets of *Streptochaeta* are complex, featuring several bract-like organs rather than lodicules or paleas. Some of these organs express B-class genes, suggesting they are homologous to floral organs despite differences in character (Whipple et al., 2006). However, these data do not help clarify lemma homology. Further gene network analysis in *Streptochaeta*, building on existing data for specific genes (Preston et al., 2009; Reinheimer and Kellogg, 2009), including additional sampling from close grass relatives, would be valuable.

Other developmental comparisons within the grasses have been more informative. Historically, morphologists generally agreed the lemma is homologous to a bract,

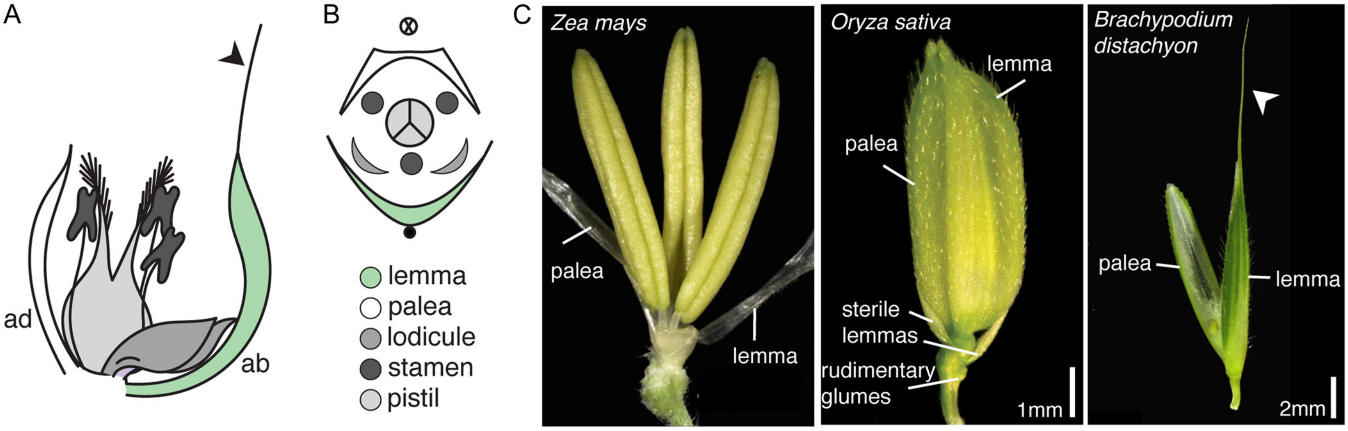

FIGURE 1 Grass floral anatomy. (A) Simplified grass flower. (B) Generic grass floral diagram. Black arrowhead in A and solid black circle in B denote awns. (C) Floret of *Zea mays* (maize), spikelet of *Oryza sativa* (rice), floret of *Brachypodium distachyon*. Arrowheads denote awns.

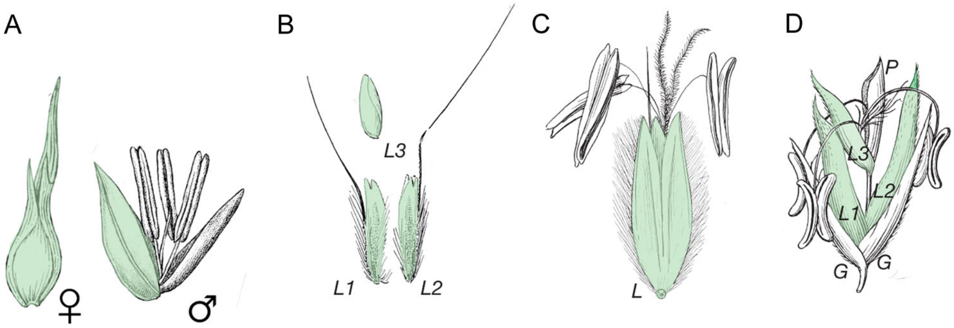

FIGURE 2 Floral diversity in grasses. (A) *Buchloe dactyloides*, lemma from female (left) and flower from male (right) plants. (B) *Anthoxanthum odoratum*, awned lemmas of sterile flowers (lower, L1 and L2), and unawned fertile flower (above, L3). (C) *Alopecurus pratensis*, flower from adaxial view, fused lemma, palea absent. (D) *Cynosurus cristatus*, spikelet with two sterile lemmas (L1 and L2) and one fertile lemma (L3). G = glume, L = lemma, P = palea. Images from Hitchcock-Chase Collection of Grass Drawings, on indefinite loan from the Smithsonian Institution, courtesy of Hunt Institute for Botanical Documentation, Carnegie Mellon University, Pittsburgh, PA, USA.

rather than to a sepal, primarily because the lemma develops on the spikelet's main axis, subtending the flowers, which develop from meristems in the axils of the lemmas, and is therefore not part of the flower (Chapman, 1996; Kellogg, 2000). The development of the lemma on the main axis fulfills Remane's first criterion of equivalent position, indicating that lemmas are homologous to bracts because each subtends a meristem. Awned lemmas have also influenced hypotheses about lemma homology, as they are thought to be homologous to grass leaves (Chapman, 1996), with the lemma body homologous to the sheath, and the awn to the blade (Figure 3) (Duval-Jouve, 1871). Lemmas and bracts have remarkably similar anatomy, with some species' glumes and lemmas almost identical (such as those of *Briza* spp., *Festuca* spp., among others), fulfilling Remane's second criterion of structural similarity.

MUTANTS AND GENOMICS SUGGEST MORE COMPLEXITY

The expression pattern of a single gene is not sufficient to define homology. However, entire genetic networks are usually affected in single-gene mutants. Thus, single-gene mutants, especially of homeotic genes that may be central to ChINs, can be valuable in defining homology. There are maize, barley, and wheat mutants in which lemmas are converted to leaves or glumes rather than floral organs (Colasanti et al., 1998; Pozzi, 2002; Debernardi et al., 2017). These "transitional states" address Remane's third criterion and suggest that lemmas are homologous to bracts.

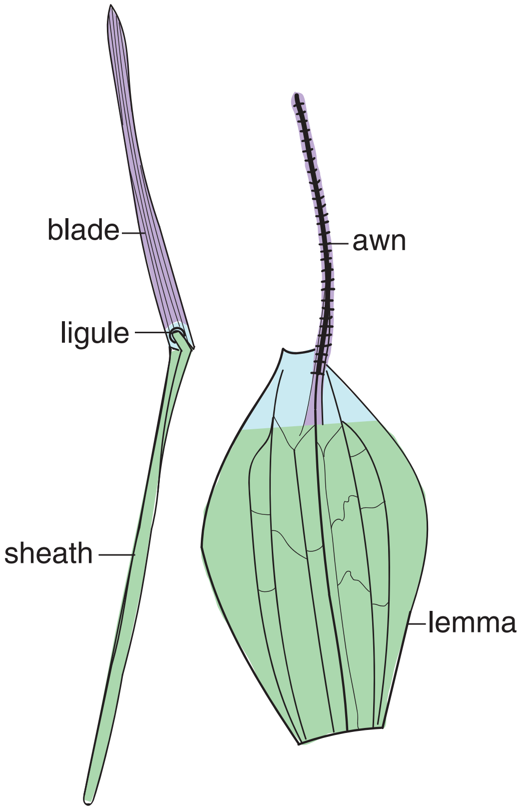

FIGURE 3 Putative homologous regions between grass leaf and awned lemma. Green, leaf sheath and main lemma body; blue, leaf ligule and lemma tissue distal to awn insertion point; purple, leaf blade and awn. Not to scale, redrawn from Thi-Tuyet-Hoa (1965).

Mutants of floral organ identity genes in rice and maize have led to a contrasting interpretation. B-class MADS-box gene mutants in maize and rice are described as having lodicules homeotically converted to "lemma/palea-like structures". Because B-class mutants in eudicots have petals homeotically converted to sepals, these grass mutant phenotypes have been used as evidence that the lemma and palea are homologous to sepals (Lombardo and Yoshida, 2015). However, rice and maize paleas and lemmas are morphologically similar (Figure 1C), complicating the interpretation of mutant phenotypes. In species with morphologically distinct lemmas and paleas, B-class mutants would better inform homology definitions: if lodicules are transformed to paleas, paleas alone are likely homologous to sepals. Thus, genetic analyses in species where the lemma and palea are distinct, such as many species in the Pooideae, would be informative.

The expression patterns of putative floral organ identity genes in diverse grasses and grass relatives suggests a more complex lemma identity than direct sepal homolog (Preston et al., 2009). In particular, A- and E-class MADS-box genes have expression patterns that extend beyond floral organs to include lemmas and glumes (bracts). If these genes are floral organ identity genes in the grasses, these data suggest that the grass lemma represents a novel organ between bract and sepal identity (Reinheimer and Kellogg, 2009), but would benefit from additional genetic analyses in diverse grasses and determination of grass ChINs. If, for instance, ChINs for bract and sepal identity were defined, they could be compared to genes involved in lemma development to determine whether lemmas rely on the leaf ChIN, sepal ChIN, or a combination of both.

New technologies can help define homologies: single-cell RNA sequencing, which analyzes gene expression in thousands of cells across spatial and temporal gradients, has revealed genetic underpinnings of developmental trajectories in rice spikelets. Despite morphological similarity of lemmas and paleas in rice, lemma gene expression is similar to that of glumes, while palea gene expression is similar to that of lodicules and reproductive organs (Zong et al., 2022). This expression pattern again suggests that lemmas, with gene expression patterns more similar to bracts, may be homologous to bracts. Thus, even without defined ChINs, transcriptomes can provide information about developmental networks likely underlying organ identities. Genetic and transcriptomic data from a broader swath of grass morphological diversity could help to define ChINs and further clarify homologies.

INTEGRATING MORPHOLOGY AND MOLECULES IN EVO-DEVO

Floral complexity does not always fit within paradigms that are based on the most well-studied clades. Even within the grasses, where flowers are relatively simple, understanding homology is challenging. Homology assessments require the careful integration of both genetic and morphological data. While homology is often a useful concept, it primarily provides a hypothesis with which to shape our research questions. Indeed, it may be that lemmas cannot be defined as distinct organ types. Instead, they may be on a continuum between bracts and sepals, borrowing identity genes from each developmental program (Kellogg, 2000). Biological complexity makes organismal traits difficult to neatly categorize. We should be open to gray areas, novelty, and times when our externally imposed categories may not reflect biological reality, while integrating information from as many sources as possible, from morphology to molecules.

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