IBC2017 General Symposium T2:22
16:00-18:00, July 28 (Friday) in Shenzhen Convention & Exhibition Center, Shenzhen, China

Mechanical forces in flowers: An evolutionary perspective

Organizers:

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Purpose of symposium

Physico-mechanical forces on shoot apical meristems have been considered a crucial key for the understanding of morphological development in plants. However, studies in flowers, including floral development, tend to neglect the importance of such forces on shaping morphological diversity and floral evolution. Flowers as growing physical entities are subjected to continuous or sequential forces during their development that define their shape and number of parts. These forces act independently or in agreement with the genetic basis of flower morphogenesis. On the other hand, as sexual devices, flowers are highly exposed to morphological variation regulated by their interaction with pollinators. New technologies and interdisciplinary collaborations have made it possible to characterize the mechanical dynamics and their influence on cellular and developmental processes in the floral apex. Perceiving how and why morphological variations in flowers respond to such forces is a challenging contribution to unveil constraints behind flower diversity as well as to complement current scientific knowledge. This symposium will focus on the important role of mechanical forces in floral evolution by revisiting floral developmental patterns of key plants, and its coordination with gene expression. We will also discuss how morphological variation induced by mechanical forces influences pollination. Finally, we will also approach the mechanical dynamics on a cellular basis in the floral apex and reveal the coordination between mechanical forces and auxin distribution. This symposium is organized by the FLO-RE-S (FLOwer REsearch Synectics) network, which aims to promote discussion and research on the question about the morphological nature and evolution of flowers.

1. Forces in flowers and their evolutionary significance

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Though many flowers demand physical force from their pollinators little is known about the functional and evolutionary significance of barriers in flowers. Among many open questions, two are of particular importance: Are weak insects excluded from pollen transfer? And do low forces have a selective significance? To get an insight into force dimensions, we measured floral forces with a highly sensitive force measurement device. We selected owers with a force demanding pollination mechanism from six angiosperm families: Lamiaceae: *Salvia* (> 30 ssp.), Zingiberaceae: *Roscoea* x *beesiana*, Fabaceae: 11 genera (13 ssp.), Polygalaceae: *Polygala myrtifolia*, Calceolariaceae: *Calceolaria* (4 ssp) and Asclepiadaceae: *Asclepias* (3 ssp.). Results fall into two groups: In Fabaceae, forces range from very low values up to more than 200mN. Compared to published data on bee forces, it is evident, that weak insects are excluded by some keel flowers. Surprising- ly, in all other flowers, forces are rather weak not exceeding an average of 15 mN. They do not exclude bees from pollen transfer completely, but may have a functional significance during food plant selection. To test the functional significance of low forces, we constructed artificial '*Salvia'* flowers, ie a flower model with a lever-like barrier and a control model without any barrier.

We trained honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) to the models and found, that both bee species were able to discriminate low forces They preferred the model with no barrier as long as nectar rewards were equal. However, choice experiments indicated that the bees preferred the model with barrier when it offered a higher concentrated sucrose solution. Thereby, bumblebees were more successful on models with barriers than honeybees. We preliminarily conclude that flowers demanding low forces may play a selective role relative to the environmental conditions of the plant species In the presence of competing bee species, they may contribute to niche formation by offering stronger bees a nectar source with reduced competition.

2. The impact of floral developmental constraints on the evolution of flowers

Louis Ronse De Craene

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Flower development and shape is regulated by the interaction of a genetic program and environmental factors, such as influence of pollination systems and biophysical forces acting on developing flowers The initiation of floral meristems and floral organs is mainly regulated by spatial constraints. Three major factors are responsible for creating these spatial constraints and influence the morphology of flowers: time, size, and pressure. Changes in morphology are mainly induced by shifts in time of initiation of organs, pressure of previously initiated organs, and alterations of the size of the floral meristem, and these operate independently of genetic factors. A number of examples demonstrate this inter- action and its importance in the establishment of different floral forms

Delays in the timing of initiation of organs lead to the establishment of novel morphologies by absorption of tissue, such as in stamen-petal primordia, or by inducing shifts of organ positions, such as the phenomenon of obdiplostemony.

Floral meristem expansion will provide more space and will lead to an increase of stamen and carpel number, the development of hypanthia and accessory structures, and changes in merism. Merism is mainly established by a balance between the size of organs versus the size of the floral apical meristem.

Pressures can act centripetally by the action of external organs, or centrifugally by the inner meristem size directing the number and arrangement of organsIt is demonstrated that changes in the physical environment of apical meristems has an important influence on ontogenetic processes leading to changes in floral configurations and major shifts in floral form, which is reflected in the phylogeny.

3. Spatial constraints influence the shape of spikelets and flowers in Cyperoideae (Cyperaceae)

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Cyperaceae is a large grass-like family in Poales with two sub- families, the small Mapanioideae and the species-rich Cyperoideae, each subfamily corresponding to a well-supported sister clade. Based on scanning electron microscopic observations we studied the development of the flowers and inflorescences of representatives in a wide range of genera of Cyperoideae

Cyperoideae are characterized by spikelets composed of wind-pollinated, typically trimerous, monocotyledonous flowers. The spikelets are the units of compound inflorescences, namely panicles of spikelets. The panicles are often compacted and contracted into dense head-like inflorescences. In such inflorescences, spatial constraints are obviously influencing the development of the spikelets as well as the individual flowers.

Reduction of the number of flowers in the spikelet, loss of the spikelet prophyll, metatopic displacements of the bracts subtending a flower, and reduction of the floral parts of the flowers are the main consequences of spatial constraints.

Within the flowers, spatial constraints may result in the reduction of the number of stamens to three instead of six, delaying or totally reducing the development of the abaxial floral parts, the loss of the perianth and modifications of the gynoecium. In the most derived subtaxa of Cyperoideae, the compacted inflorescence tends to take over the floral function, forming dense heads consisting of single-flowered spikelets. The question remains whether these evolutionary trends were driven by mechanical forces or by pollination strategies, or a combination of both.

4. Development of actively morphing structures in plants

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Throughout the phylogeny of seed plants, flowers and fruits contain actively moving structures to facilitate pollination or seed dispersal. Examples include pollinator-induced movement of stamens and/or carpels, diurnal flower opening, and moisture-con- trolled dispersal of pinecones and wheat awns. These functional structures require unique cellular and tissue-level features that are precisely patterned and generated during development. In this talk I will present an overview of the biomechanics behind the moving parts in flowers and fruits and highlight the commonly found morphological and anatomical strategies. By understanding better about the hydrodynamic and tissue material features under- lying the actively controlled organ movement, we can identify the candidate key developmental events that are also likely to have been targeted by the evolutionary innovation of such morphing structures. I will then report our effort to establish an experimental platform to untangle the physico-chemical feedback loops regulating plant organ development, which can be used to test hypotheses generated above.

5. Forces in flower ontogeny

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From an evolutive perspective, the flower can be understood as the historical outcome that serves reproduction and maintenance of the plant's lineage. At the same time, but from an organismic point of view, the flower can be seen as the outcome of the ontogenetic process in each individuum. Questions regarding the ontogenetic process of the flower involve multiple aspects including cell proliferation and growth, hormone flux, gene expression patterns, as many others. One of these elements that has received undeservedly little attention are mechanical forces itself. In this work we present a revision of a number of examples where mechanical forces have been shown to play a fundamental role in shaping the flower at different moments throughout ontogeny. The palette of action of these forces go from the early ontogeny at the microscopic level, - as the organ initiation patterns on the flower meristem in relation to the pressure exerted by inflorescence axes or neighboring primordia -, to the macroscopic level in the late ontogeny, as the twisting of a pedicel of a resupinating flower. The premise here conceived is that without these forces occurring during ontogeny, the recalled flower phenotype wouldn't be ex- pressed as it is actually known, implying that the mentioned force plays a causal-generative role on the flower's phenotype, unaware of further genetic or molecular response cooccurrence.

6. Pollinator-driven floral evolution with and without pollinator shifts in *Impatiens* (Balsaminaceae)

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Floral diversity may evolve as a result of plant-pollinator interactions by two distinct processes: through the use of different functional pollinator groups, or divergent use of the same pollinator. However, the relative importance of these processes is largely unknown. Here we use the recently

radiated, large plant genus *Impatiens*, characterized by variable, zygomorphic or asymmetric flowers, to understand the association between floral diversity and pollination ecology, and to study its evolution. A comparative field study of co-flowering *Impatiens* species in northern Thailand revealed that out of seven species, six are dependent on animal visits for pollination. Several different types of animals visited the flowers, but not all were effective pollinators. Four *Impatiens* species are pollinated by bees, whereas two species are pollinated by bees and butterflies. The main features that distinguish pollination systems included the shape and dimensions of the floral chamber and length of the (nectar) spur. Co-flowering species that shared bee pollinators deposited their pollen on different parts of the bee body, including through an unusual form of the asymmetric perianth. Based on our field-observations we predicted pollinators at the level of functional groups for a large number of *Impatiens* species. The majority of species was inferred to be bee-pollinated, and floral asymmetry was significantly more common among bee-pollinated species than those with other pollination systems According to the phylogenetic analysis, shifts between functional pollinator groups occurred at least 17 times. Floral asymmetry evolved at least 11 times, never reversed, and was not associated with large clades. Shifts to floral asymmetry were uncoupled from shifts between functional pollinator groups, and always happened in lineages that were ancestrally bee-pollinated. These results are consistent with a scenario according to which floral diversity either evolves by means of pollinator shifts, or by divergent use of the same, frequently exploited pollinators. Once floral asymmetry evolves it does not appear to function as key-innovation that allows species to invade a new niche leading to clade proliferation Further analyses, which take the co-occurrence of *Impatiens* species into account, could reveal whether competition between co-flowering species is an important driver of floral evolution.