

Review and Advances in Style Curvature for the Malvaceae

Cheng-Jiang Ruan*

Key Laboratory of Biotechnology & Bio-Resources Utilization, Dalian Nationalities University, Dalian City, Liaoning 116600, China

Correspondence: * ruancj@yahoo.com.cn

ABSTRACT

The flowers of the Malvaceae with varying levels of herkogamy via style curvature have long intrigued evolutionary botanists. This review covers the flower opening process, approach herkogamy, style curvature and character evolution based on molecular phylogenetic trees, adaptive significances of style curvature and the mating system in some portions of the genera in this family. Hermaphroditic flowers of some species have showy petals and pollen and nectar rewards to pollinators. Approach herkogamy, in which stigmas are located on the top of a monadelphous stamen, has evolved as a mechanism to reduce the frequency of intra-floral self-pollination or the interference between male-female organs. Protandrous or monochogamous flowers in the fields open at about 5-7 days and 1-2 days respectively, and pollination is conducted by insects and birds. Interestingly, un-pollinated styles in some species curve when pollination fails. According to our observations and published or internet data, this curvature occurs in 23 species distributed in eight genera of four tribes (Malvaceae, Urenaee, Hibisceae, Malveae) and appears to have evolved at least eight times. A shift to use style curvature is associated with a shift to annual or perennial herbs, and an unpredictable pollinator environment is likely an important trigger for this evolution. The adaptive significances of style curvature in the Malvaceae include delayed selfing, promotion of outcrossing or reduction in intrafloral male-female interference, sometimes two or three of which simultaneously occur in style curvature of one species (e.g., *Kosteletzkya virginica*). Delayed selfing via style curvature represents reproductive assurance and a mixed mating system, but the relationship between them is still an evolutionary enigma.

Keywords: adaptive significance, character evolution, floral structure, mating system, phylogenetic approach, reproductive assurance

CONTENTS

INTRODUCTION.....	98
FLORAL MORPHOLOGY AND POLLINATION BIOLOGY.....	99
Floral structure.....	99
Flower opening process.....	99
Approach herkogamy.....	99
Pollination biology.....	101
STYLE CURVATURE AND COROLLA CLOSURE.....	102
Style curvature.....	102
Corolla closure promotes delayed selfing.....	102
EVOLUTION OF STYLE CURVATURE AND ITS CORRELATED CHARACTERS.....	103
Evolution of morphological characters.....	104
Evolution of style curvature and correlated traits.....	106
Evolutionary relationship of style curvature and plant type-habit.....	106
Unpredictable pollinator environments triggering the evolution of style curvature.....	106
Biogeographical history of style curvature.....	107
ADAPTIVE SIGNIFICANCE OF STYLE CURVATURE.....	107
Adaptive significance.....	107
Promotion of outcrossing.....	107
Delayed selfing pollination.....	107
Reduction in intrafloral male-female interference.....	107
Reproductive assurance.....	108
Mixed mating system.....	108
CONCLUSION.....	109
FUTURE RESEARCH.....	109
ACKNOWLEDGEMENTS.....	109
REFERENCES.....	109

INTRODUCTION

Since Darwin (1862) observed reconfiguration in orchid pollinia, the dramatic movement of floral structures in many angiosperms, which influence plant pollination and mating systems, has long intrigued evolutionary botanists (Buttrose *et al.* 1977; Faegri and van der Pijl 1979; Schlessman 1986;

Juncosa and Webster 1989; Dole 1990; Lyon 1992; Rathcke and Real 1993; Donnelly *et al.* 1998; Eckhart and Schaeffer 1998; Traveset *et al.* 1998; Fetscher and Kohn 1999; Kalisz *et al.* 1999; Kennedy 1999; Bynum and Smith 2001; Fetscher 2001; Li *et al.* 2001; Wang *et al.* 2004; Edwards *et al.* 2005; Liu *et al.* 2006; Peter and Johnson 2006). Meanwhile, the reproductive assurance of maximum seed production

and the evolutionary relationship between them presented by floral movements in response to unpredictable pollinator environments has always been an evolutionary enigma to the mixed mating system (Herlihy and Eckert 2002; Kalisz *et al.* 2004; Goodwillie *et al.* 2005; Cheptou and Schoen 2007). The interesting curvature of un-pollinated styles towards the anthers is known to occur in several species of the Malvaceae family, including *Hibiscus trionum* (Buttrose *et al.* 1977), *Hibiscus laevis* (Klips and Snow 1997) and *Kosteletzkya virginica* (Ruan *et al.* 2005a), which may involve different adaptive significances that include achievement of delayed selfing (Blanchard 1976), promotion of outcrossing (Stephens 1948), and supplies for outcrossing the highest priority, while retaining the ability to self if outcrossing fails (Buttrose *et al.* 1977).

There are about 100 genera and *ca.* 1000 species in the Malvaceae all over the world, which is distributed in tropical and temperate regions of the Northern and Southern Hemispheres. How and why members of the Malvaceae are distributed as they are has long puzzled evolutionary botanists. Floral structure with an approach monadelphous column and varying herkogamy (HK) via style curvature (see the appendix I of glossary of terms) in some species, which indicated mixed mating and reproductive assurance of maximum seed production, presents an idea evolution and dichotomy (Stephens 1948; Buttrose *et al.* 1977; Klips and Snow 1997; Ramesy *et al.* 2003; Ruan *et al.* 2004, 2005a, 2005b, 2005c; Seed *et al.* 2006; Ruan *et al.* 2008; Ruan *et al.* 2009a, 2009b, 2009c). Malvaceae is always at a core location of the Malvales in differently taxonomic systems (e.g. Linnaeus and Jussieu), but it is still unclear to the circumscriptions among different families in the Malvales, especially between the Malvaceae and the Bombacaceae (Bayer *et al.* 1999; Wu *et al.* 2004). For example, Edlin (1935) moved the tribe Hibiscieae and the *Kydia* genus into the Bombacaceae, but Hutchinson (1967) argued that "Malvaceae without the great genus *Hibiscus* would be like a horse without a tail" (Takhtajan 1997). Furthermore, morphological diversity, which was used in classified circumscriptions of the species, the genus and the tribe, leads to a nomenclatural and classification dilemma within the Malvaceae. Recently, increasing molecular studies are providing the potential for the circumscription of different the genera and species (Seelanan *et al.* 1997; Baum *et al.* 1998; Alverson *et al.* 1999; Pfeil *et al.* 2002; Baum *et al.* 2004; Small 2004; Koopman and Baum 2008), and have shown that the members of the Bombacaceae, Malvaceae, Sterculiaceae, and Tiliaceae form a very well-defined monophyletic group that is divided into 10 also rather well-defined clades, only two of which correspond to the traditional families Bombacaceae and Malvaceae. These published molecular phylogenetic trees provide the potential to analyze the occurrence of style curvature at the family level.

Here, I first present (i) the flowers opening process and pollination biology for some species in the Malvaceae, (ii) approach HK and its variation within or among some species within the family, (iii) the curvature of un-pollinated styles and its distribution in 52 species of 13 genera of the Malvaceae, and (iv) the evolution of morphological characters (e.g., fruit type, filament tubes with anthers insert along length or at apex, confluent or divided styles, style branches as many as ovary locules or *ca.* 2 × as many as carpels, stigmata shape, ovule number per ventricle and epicalyx numbers), style curvature and its correlated characters. Then, I discuss possible adaptive significances of style curvature and a mixed mating system in the Malvaceae. Finally, for future research, I suggest that more studies be carried out on the pollination and mating systems of the Malvaceae in a wider species of different genera, and that phylogenetic research should be conducted to understand the evolution of pollination and mating systems in the Malvaceae. For style curvature, more detailed studies should be done about the relative importance of its different adaptive significances and their harmonious mechanism.

FLORAL MORPHOLOGY AND POLLINATION BIOLOGY

Floral structure

The Malvaceae contains herbs, shrubs, or less often trees, and usually have an indumentum with peltate scales or stellate hairs (Tang *et al.* 2007). Leaves are alternate, stipulate and petiolate (e.g. leaves of different types of *K. virginica*, **Fig. 1A**); the palmate leaf blade is usually veined, entire or various lobed. Flowers are usually hermaphroditic and entomophilous, unisexual in *Kydia* (Tang *et al.* 2007) and female flowers are rare (vestigial stamens, **Fig. 1B**) in *K. virginica* (Ruan *et al.* 2005c). An epicalyx is often present, forming an involucre around the calyx with 3- to many-lobed. Valvate 5-sepals are free or connate. Five petals are free, contorted, or imbricate, basally adnate to the base of the filament tube. Obvious floral characters in the Malvaceae differing from other families is its monadelphous column (e.g. *Hibiscus syriacus* in **Fig. 1C**), in which filaments are united to form one set or tube, high or low anthers forming part of the tube are placed below the stigmas. Anthers are one-celled, with spiny pollen. The ovary is superior, with 2-25 carpels, often separating from one another and from the axis; ovules are 1 to many per locule. Style has branches (**Fig. 1D**) or no (**Fig. 1E**). The number of styles is as many or 2× as many as pistils, with the apex branched or capitate. Fruit is a loculicidal capsule (**Fig. 1F** and **1G**) or a schizocarp (**Fig. 1H**), usually separating into individual mericarps when mature (rarely berry-like in *Malvaviscus*). Seeds (**Fig. 1I**) are often reniform or obovate, and glabrous or hairy, with an endosperm.

Flower opening process

Hermaphroditic flowers of the Malvaceae are protandrous or monochogamous. To observe the flower opening process, I selected the *Althaea rosea*, *K. virginica* and *H. trionum* as the samples in the fields. For observed species, we recorded the flowering period of over 20 flowers, including mature time of male-female organs, position of stigmas, anther dehiscence, etc.

According to the position of stigmas relative to the monadelphous column after the flowers fully open and the separation of maturing time of pistils and stamens, the flower opening process may be classed as one of two types: one is a single protandrous flower that opens within about 5-7 days. Anthers dehisce when the flowers are fully open, but at that time style branches do not exert out of the monadelphous column (e.g. *Althaea rosea* in **Fig. 1J**). After flowers open within 1-2 days, most pollen sheds, style branches grow out of the monadelphous column (e.g. *Althaea rosea* in **Fig. 1K**), the flower begins the female stage. This flowering process occurs in some species of the tribe Malvaeae, such as *Althaea rosea*, *Anoda cristata*, *Malope trifida*, *Malva neglecta*, etc. Another is the single monochogamous flower only open one day (rarely two days), style branches protrude out of the monadelphous column when the flower buds form and the flowers are fully open (e.g. *Kosteletzkya pentacarpos* in **Fig. 1D**). This flowering process occurs in common species except for the Malvaeae, such as *K. virginica*, *Hibiscus trionum*, *Hibiscus surattensis*, etc.

Approach herkogamy

The monadelphous column in the Malvaceae segregates male and female reproductive organs, forming HK (the spatial separation of pollen presentation and pollen receipt within a flower) (Ruan and Jiang 2007). The HK is directly influenced by whether filament tubes with anthers insert at the apex of the monadelphous column: (i) filament tubes insert at the apex, there is no or small HK occurring in some species of the tribe Malvaeae, such as *Abutilon hybridum*, *A. pictum* (**Fig. 1L**) and *A. theophrasti*; and (ii) filament tubes

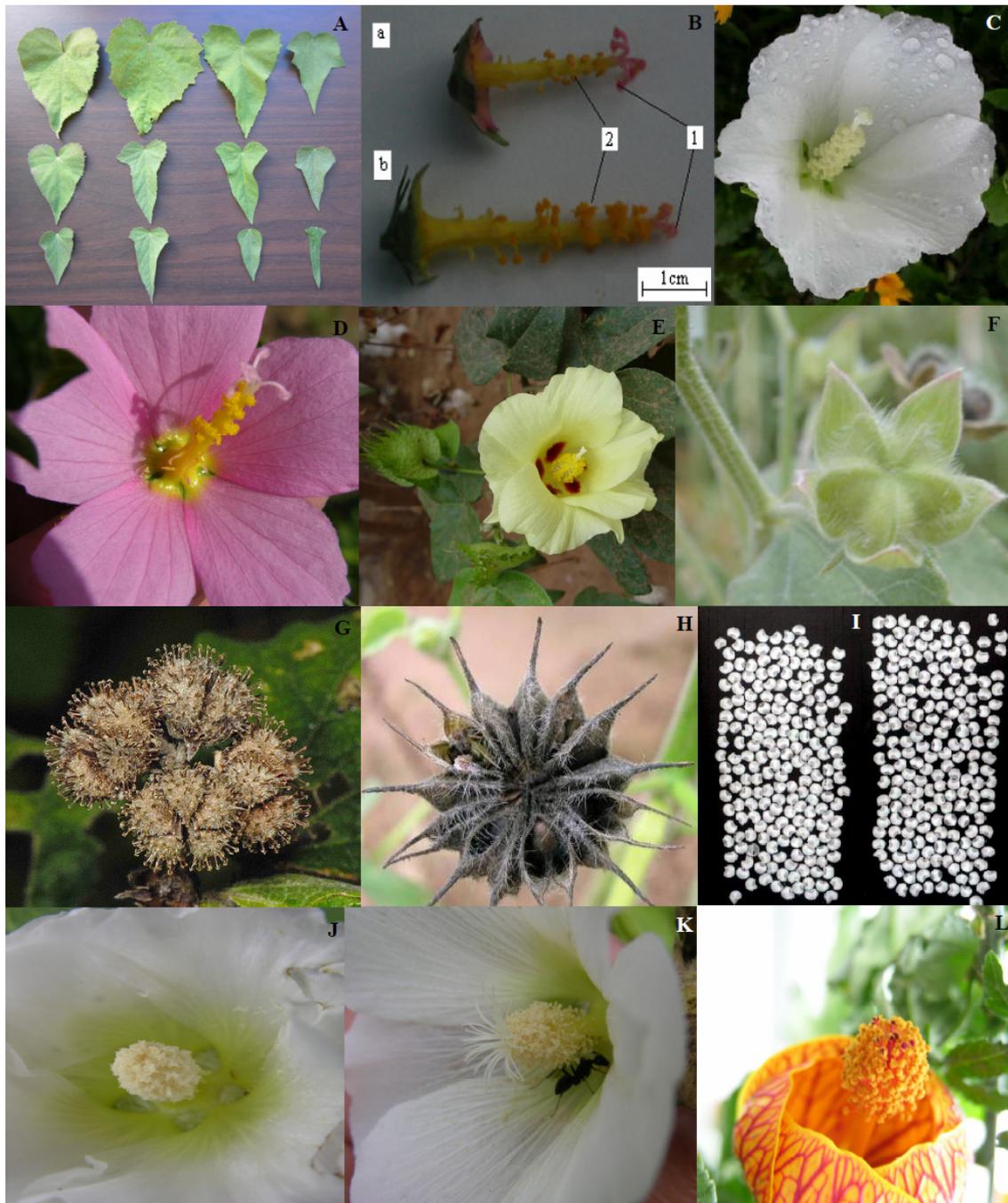


Fig. 1 (A) Leaves of different types of *Kosteletzkya virginica*. (B) Sex dimorphism in *K. virginica*, in which (a): female flowers, stamens are vestigial; (b): hermaphroditic flowers, stamens are well developed and fertile. 1: stigma; 2: stamen. (C) Monadelphous column (MC) in the flower of *Hibiscus syriacus*. (D) Monochoгамous flower of *Kosteletzkya pentacarpos*. (E) Flower of *Gossypium hirsutum*. (F) A capsule of *K. virginica*. (G) A split capsule of wild cotton. (H) A schizocarp of *Abutilon theophrasti*. (I) X-ray image of seeds of *K. virginica*. (J) Male stage of protandrous flower of *Althaea rosea*, stigmas hide within the monadelphous column after flowers are fully open. (K) Female stage of protandrous flower of *A. rosea*, stigmas exert out the column after pollen is shed after 1-2 days. (L) Flower of *Abutilon pictum* without herkogamy. All photographs by C.-J. Ruan except for (G), which is from: http://images.google.cn/imglanding?imgurl=http://farm3.static.flickr.com/2172/2216401736_47bd28fetc.jpg%3Fv%3D0&imgrefurl=http://flickr.com/photos/17457752%40N00/2216401736&usq=__wtmiLIHVBIS4fj4w6zSvuHRZ-fa%3D&h=363&w=500&sz=130&hl=zh-CN&um=1&tbnid=k_xrn-kk52v3PM:&tbnh=94&tbnw=130&prev=/images%3Fq%3D%25E6%25A3%2589%25E8%258A%25B1%25E6%259E%259C%26h1%3Dzh-CN%26sa%3DN%26um%3D1%26newwindow%3D1&q=%E6%A3%89%E8%8A%B1%E6%9E%9C&sa=N&um=1&newwindow=1&start=2#start=5

insert along sides, indicating approach HK, such as *K. virginica* (HK = 3.31 ± 0.09 mm), *H. trionum* (HK = 4.12 ± 0.09 mm) and *H. syriacus* (HK = 9.53 ± 0.30 mm) (Zeng *et al.* 2008).

Much more attention has been paid to the hypothesis that HK has evolved as a mechanism to reduce the frequency of intra-floral self-pollination (Darwin 1876; Holtsford and Ellstrand 1992; Brunet and Eckert 1998), specifically in Malvaceae studies (Ruan *et al.* 2009c). This is because there is a positive correlation between HK and outcrossing rates in some species with mixed-mating systems (Belaous-

soff and Shore; Karron *et al.* 1997; Motten and Stone 2000), and exemplifications that HK influences self-pollination (Klips and Snow 1997; Miyajima 2001; Elle and Hare 2002) and inbreeding depression (Stone and Motten 2002). In the Malvaceae, cytogenetic characteristics confirmed that *H. acetosella* and *H. cannabinus* are outbreeders, while *H. asper*, *H. physaloides*, *H. sabdariffa* and *H. surattensis* have evolved into inbreeders (Akpan 2000). The inbreeding species appear to have co-evolved (a strong assertion of co-evolution) a floral structure in which some anthers abut on the stigma prior to anthesis (no or small HK) (Akpan 2000).

Spira (1989) concluded that HK effectively prevents self-pollination in self-compatible *H. moscheutos*, but does not prevent geitonogamy (Snow *et al.* 1996). When visiting a flower with approach HK, the pollinators often land on the upturned stigmas and then proceed towards the base of the flower by wading through the numerous pollen-laden anthers. Departure from the flower is usually from the flower petals so that the stigmas are not touched again (Ruan and Jiang 2007).

In contrast, increasing evidence suggests that HK may act as a mechanism to reduce interference between the reproductive functions of female and male organs (Webb and Lloyd 1986; Bertin and Newman 1993; Barrett 2003). Studies of heterostylous species indicate that sex-organ reciprocity is a mechanical device increasing the proficiency of cross-pollination as a result of segregated pollen deposition on different parts of the bodies of pollinators, and that enantiostyly and stigma-height dimorphism also function in a similar manner (Jesson and Barrett 2002; Thompson *et al.* 2003). In the Malvaceae, approach HK varies among species (Zeng *et al.* 2008) and within a species (Ruan *et al.* 2009c), for example it ranged from 0.9 to 7.4 mm in *K. virginica* (Ruan *et al.* 2005c) and from 0.98 to 1.24 cm in *H. syriacus* (Zeng *et al.* 2008). Separation of male and female organs (HK) may reduce interference between male-female organs (Zhang 2004) and the frequency of intrafloral self-pollination (Webb and Lloyd 1986; Lloyd and Schoen 1992). However, in the herkogamous flowers, too great a distance between stigmas and anthers reduces the probability that pollinators will be in contact with both sexual organs when visiting, and may decrease the veracity of cross-pollen transfer and male-female fitness (Zhang 2004). For example, curvature of un-pollinated stigma lobes results in autogamous selfing in *H. laevis* plants from northern populations more than in those from southern populations; this is because the distance between HK before the curvature of un-pollinated lobes for south populations is too great to allow selfing in plants (Klips and Snow 1997). Additionally, too great HK often leads pollinators with a small body to only contact pollen when the pollinators visit flowering (Spira 1989; Spira *et al.* 1992; Ruan *et al.* 2010).

Pollination biology

Pollination has long fascinated evolutionary botanists, in which pollination biology of different species in the Malvaceae is also widely considered (Weiss and Dickerson 1919; Fernald 1942; Spira 1989). For the Malvaceae, wind pollination is unlikely as the pollen grains are sticky and tend to clump together. Flowers of *H. moscheutos* covered with a single layer of cheesecloth (porous to pollen but not to insect pollinators) accumulated few to no pollen grains on stigmas and failed to mature fruits, indicating that flowers were not apomictic and that a vector other than wind was needed for successful pollination (Spira 1989). Ruan *et al.* (2005a) also noted that *K. virginica* is not agamospermous. Hence, many species of the Malvaceae are entomophilous, with the large-hollyhock-like flowers that have a large corolla size and showy colors (common pink, white, purple, red, etc.) attractive to pollinators, and with nectar and pollen rewards to pollinators (Feng 1984). For example, the two pollinator species, *Bombus* and *Ptilothrix* of *H. moscheutos*, use petals as a cue to locate *Hibiscus* flowers because flowers with 100% petal removal are almost completely ignored (Kudoh and Wigham 1998). Pollinators of the Malvaceae include generalized pollination insects (e.g. bees, butterflies, etc.), hawkmoths for *Kosteletzkya virginica* (Fig. 2A), hummingbirds (*Phaethornis superciliosus* for *Pavonia dasypetala*) and other birds (e.g. bananaquits and Bahama woodstars for *Pavonia bahamensis*, Rathcke 2000).

In naturalized populations of *K. virginica* growing in Jiangsu Yancheng tideland of China, several insects (e.g. wasps, butterflies, moths, etc.) were engaged in pollination (Fig. 2B-K). Common and little bees are main pollinators.

Big bee, little-worm bee, white butterfly and one species of ants are fluctuating vectors. However, I also found that self-pollination of some flowers could be conducted by wind brush pollination (Fig. 2L) when there is wind. When the wind flaps the branches with open flowers, contact between leaves and pollen leads some pollen to stay on the surface of leaves; after this, wind flaps the branches easily to make stigmas to touch this pollen, achieving self-pollination. In my observation of 4 days (from 8:00 to 16:00 for each day) at the *K. virginica* population in 2005, 5.43~15.12% of 230 flowers were pollinated by wind brush pollination.

For *H. moscheutos*, the effective pollinators are solitary anthophorid bees (*Ptilothrix bombiformis*) and bumblebees (*Bombus* spp.) (Blanchard 1976; Spira 1989; Snow and Spira 1993). The appearance of *Ptilothrix bomiformis* and disappearance of its adult is largely coincidental with the flowering of *H. moscheutos*, with much of the bee's activity centering around these plants (Blanchard 1976). Other visitors to flowers noted are several species of moths, butterflies, small bees and flies, but none appear to be effective pollinators (COSEWIC 2004). Spira (1989) and Spira *et al.* (1992) concluded that most visits by *P. bombiformis* and *Bombus* did not result in pollination, as the visitor failed to make contact with any of the flower's stigmas. In their studies only 27% of flower-foraging *P. bombiformis* and *B. pennsylvanicus* appeared to contact a stigma while foraging for nectar or pollen. However, when they did contact the stigma, they generally deposited large amounts of pollen on the stigmas (up to 889 grains), and 14 times as many pollen grains as there were ovules in the ovaries.

For *Hibiscus laevis*, the nectar and pollen of the flowers attract bumblebees and oligolectic *Emphorine* bees that prefer visiting the flowers of this and similar species. In this latter group of bees, are such species as *Melitoma taurea* (the mallow bee) and *Ptilothrix bombiformis*. The caterpillars of the butterfly *Strymon melinus* (gray hairstreak) feed on the flower buds and seeds, while the caterpillars of *Pyrgus communis* (checkered skipper), the moth *Eudryas unio* (pearly wood nymph), and the butterfly *Vanessa cardui* (painted lady) feed on the foliage (http://www.illinoiswildflowers.info/wetland/plants/halberd_mallow.htm).

Female reproductive success (fruit and seed set) in *Pavonia dasypetala* (Malvaceae) was only weakly related to pollinator attentiveness (number of pollinator visits and number of pollen grains deposited on stigmas). Many flowers that were visited numerous times by hummingbirds and that received pollen in excess of the number of ovules per flower failed to produce fruits (McDade and Davidar 1984). On San Salvador Island, *Pavonia bahamensis* appears to be pollinated exclusively by two bird species, bananaquits (*Coereba flaveola*) and bahama woodstars (*Calliphlox evelynae*). This pollination dependence was dramatically demonstrated in one season when hurricanes decimated these bird populations, and pollen deposition and fruit set of *P. bahamensis* was significantly reduced (Rathcke 2000). However, the lack of pollination limitation of fruit set during two other flowering seasons and the relatively low pollen/ovule ratio (607) suggests that pollination of *P. bahamensis* by these birds is generally reliable. Flowers show traits typical for a bird pollination syndrome, except that the corolla is green. Flowers are held horizontally on the shrub, rather than vertically, suggesting that passerine birds (bananaquits) rather than hummingbirds have been the most effective pollinator and major selective agent for the floral traits in this species. Individual bahama woodstars are ineffective pollinators, depositing few or no pollen grains on stigmas per visit; however, they maintained pollination in one season when visits by bananaquits were infrequent, and they contributed to the reliability of pollination for this endemic species.



Fig. 2 Pollination of *Kosteletzkya virginica*. (A) *Manduca florestan* was captured after visiting flowers of *K. virginica*. (B-K) Different pollinators of *K. virginica*. (L) Wind brush pollination. All photographs by C.-J. Ruan.

STYLE CURVATURE AND COROLLA CLOSURE

Style curvature

As *H. laevis*, some species of the Malvaceae decrease separation between stigmas and anthers by curving the un-pollinated stigma lobes towards the anthers, as in *H. trionum* (Ramsey *et al.* 2003), *H. laevis* and *K. virginica* (Ruan *et al.* 2004). The observation and survey on fifty-two species of the Malvaceae by Ruan *et al.* (2010) and Ruan *et al.* (unpublished) showed that style curvature, that brings stigmas into contact with its own anthers, was measured in 23 species distributed in eight genera of four tribes (Malvaceae, Urenaee, Hibisceae, Malveae), including the following two types.

Type I: styles curve before pollen shedding, and flowers have highly receptive stigmas and viable pollen when curvature brings stigmas into contact with anthers. This curvature occurs in three tribes of the Malvaceae, Urenaee and Hibisceae, such as *K. virginica*, *H. trionum*, *Pavonia hastata*, *Urena lobata*, *Abelmoschus sagittifolius*, *A. manihot*, *H. coatesii*, *H. laevis*, *H. pedunculatus*, *H. moscheutos*, *H. normani*, *H. sabdariffa*, *H. surattensi* and *K. pentacarpos* (Ruan *et al.* 2010 and references therein). My observation on *K. virginica* (Fig. 3A) in the fields showed, if stigmas are pollinated, styles remain erect or cease to curve (Fig.

3B) when pollination occurs in the process of style curvature. However, if pollination fails, style branches curve downwards towards the anther (Fig. 3C), eventually bringing stigmas into contact with the anthers (Fig. 3D) and potentially resulting in self-pollination. For *K. virginica*, about 5-9 h are needed for style curvature from the beginning (about 06:00-07:00) to cessation (about 11:00-16:00).

Type II: styles curve after most of pollen is shed, and this curvature often appears in the protandrous species of the Malveae tribe, such as *A. rosea*, *Malope trifida* and *Malva neglecta* (Ruan *et al.* 2010 and references therein). In type II flowers of *A. rosea*, after having flowered for 2-3 days, style branches grow out of the monadelphous column (Fig. 3E) and begin to curve, eventually bringing some stigmas down or in contact with the unshed anthers (Fig. 3F).

Corolla closure promotes delayed selfing

Compared to the north populations of *Hibiscus laevis*, the plants from southern populations in which the distance between HK before the curvature of un-pollinated lobes is too great to allow selfing, though the style curvature decreases the HK of *H. laevis* (Klips and Snow 1997). Similarly, for *K. virginica* flowers, approach HK of over 6.2 mm is too great to allow autonomous selfing only by style curvature (Ruan



Fig. 3 (A) Fully opened flower of *Kosteletzkya virginica*, stigmas are located on the top of the monadelphous column. If pollination fails, styles curve (C) and eventually bring stigmas into contact with the anthers (D); otherwise, styles cease curving (B) after pollination. For the flowers of *Althaea rosea*, pollen shed after 1-2 days, then stigmas exert out the column and begin to curve (E), and eventually some stigmas make contact with the anthers (F).

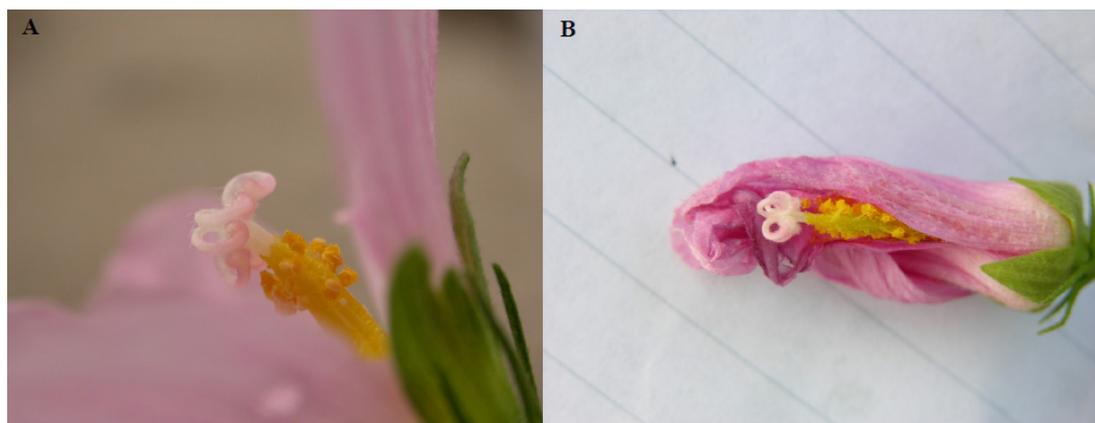


Fig. 4 Role of corolla closure in delayed selfing of the flowers of *Kosteletzkya virginica* that stigmas do not contact with its own anthers at the end of the anthesis day. (A) Corolla imminent closure about 16:00 intraday post-anthesis, in that time, stylar movement stopped and stigmas do not contact with its own anthers. (B) Corolla closure, self-pollination occurring as the gradually close corollas promoting the contact between stigmas and anthers.

et al. 2005b). In these flowers with too great HK, compared experiments between eliminated and un-eliminated corollas indicated that corolla closure, in the absence of pollinators, is a mechanism of delayed self-fertilization in this species (Fig. 4), because the fruit sets of the flowers from un-eliminated and eliminated corollas were close to 69.72 ± 1.62 and $1.68 \pm 0.36\%$, respectively (Ruan 2007). In these flowers, decrease HK via style curvature is about 1.3 mm at 16:00 (Ruan *et al.* 2005c).

EVOLUTION OF STYLE CURVATURE AND ITS CORRELATED CHARACTERS

The circumscription and classification of Malvaceae, particularly at the generic and tribal levels, are frequently disputed (Bates 1968; Fryxell 1968, 1975, 1988; Judd and Manchester 1997; Alverson *et al.* 1998, 1999; Craven *et al.* 2003). However, molecular phylogenetic studies during the last 10 years have greatly improved our understanding of

Malvaceae systematics and resulted in the recircumscription of major taxonomic groups, but also provide the potential to analyze the occurrence of style curvature at the family level. Hence, in here, I outline their uses in the evolution of morphological characters (e.g., fruit type, filament tubes with anthers insert along length or at apex, confluent or divided styles, style branches as many as ovary locules or *ca.* $2 \times$ as many as carpels, stigmata shape, ovule number per ventricle and epicalyx numbers), style curvature and its correlated characters, respectively.

Evolution of morphological characters

Some morphological traits were used in the taxonomic classification of the Malvaceae, including fruit type, filament tubes with anthers insert along length or at apex, confluent or divided styles, style branches as many as ovary locules or *ca.* $2 \times$ as many as carpels, stigmata shape, ovule number per ventricle and epicalyx numbers (Table 1). Based on the

DNA sequences (Ruan *et al.* unpublished), we analyzed their evolutionary process by mapping the trait state on the molecular phylogenetic trees reconstructed using chloroplast DNA sequences of *ndhF* and *rpl16*. The results showed that when we regarded capsule as the ancestral state, the schizocarp had evolved two times in the *ndhF* tree including seven species with schizocarp and 27 species with capsule (Fig. 5A) and one time in the *rpl16* tree including five species with a schizocarp and 35 species with a capsule (Fig. 5B). When we regarded filament tubes with anthers inserted along the length as the ancestral state, anthers inserted at the apex had evolved once in the *ndhF* or *rpl16* tree (figures of this trait evolution not shown). Similarly, when we regarded confluent styles as the ancestral state, divided styles had evolved once in the *ndhF* or *rpl16* tree (figures of this trait evolution not shown). In addition, style branches *ca.* $2 \times$ as many as carpels had also evolved one time in the *ndhF* or *rpl16* tree.

For stigmata shape, when we regarded lobed or flat stig-

Table 1 Flower characters of some species used in the evolution of morphological characters.

Family and species	FT	FTAILA	ST	RSBOL	SS	ONV	EN
Bombaceae (Family)							
<i>Pachira aquatica</i>	1	–	0	–	1	1	3
Malvaceae (Family)							
<i>Gossypoides kirkii</i>	1	1	0	–	3	1	3
<i>Gossypium hirsutum</i>	1	1	0	–	3	1	3
<i>Abutilon hybridum</i>	0	0	1	0	2	1	0
<i>A. pictum</i>	0	0	1	0	2	1	0
<i>A. theophrasti</i>	0	0	1	0	2	1	0
<i>Althaea rosea</i>	0	0	1	0	2	0	5-9
<i>Anoda cristata</i>	0	0	1	0	2	0	0
<i>Malope trifida</i>	0	0	1	0	0	0	3
<i>Malva neglecta</i>	0	0	1	0	0	0	3
<i>Sida acuta</i>	0	0	1	0	2	0	0
<i>Pavonia hastata</i>	1	1	1	1	2	0	8-14
<i>P. multiflora</i>	1	1	1	1	2	0	8-13
<i>Urena lobata</i>	0	1	1	1	2	0	5
<i>Abelmoschus ficulneus</i>	1	1	1	0	2	1	5, 6
<i>A. manihot</i>	1	1	1	0	2	1	4, 5
<i>Hibiscus calyphyllus</i>	1	1	1	0	2	1	5
<i>H. coatesii</i>	1	1	1	0	2	1	–
<i>H. coccineus</i>	1	1	1	0	2	1	12
<i>H. dasycalyx</i>	1	1	1	0	2	1	12
<i>H. divaricatus</i>	1	1	1	0	2	1	–
<i>H. engleri</i>	1	1	1	0	2	1	7-10
<i>H. fryxellii</i>	1	1	1	0	2	1	–
<i>H. furcellatus</i>	1	1	1	0	2	1	–
<i>H. heterophyllus</i>	1	1	1	0	2	1	10-12
<i>H. insularis</i>	1	1	1	0	2	1	5
<i>H. laevis</i>	1	1	1	0	2	1	10-12
<i>H. ludwigii</i>	1	1	1	0	2	1	5
<i>H. macrophyllus</i>	1	1	1	0	2	1	10-12
<i>H. moscheutos</i> ssp. <i>moscheutos</i>	1	1	1	0	2	1	10-12
<i>H. moscheutos</i> ssp. <i>incanus</i>	1	1	1	0	2	1	10-12
<i>H. moscheutos</i> ssp. <i>lasiocarpus</i>	1	1	1	0	2	1	10-12
<i>H. moscheutos</i> ssp. <i>palustris</i>	1	1	1	0	2	1	10-12
<i>H. normanii</i>	1	1	1	0	2	1	–
<i>H. pedunculatus</i>	1	1	1	0	2	1	7-8
<i>H. radiatus</i>	1	1	1	0	2	1	–
<i>H. rosa-sinensis</i>	1	1	1	0	2	1	6-7
<i>H. sabdariffa</i>	1	1	1	0	2	1	8-12
<i>H. splendens</i>	1	1	1	0	2	1	10-12
<i>H. surattensis</i>	1	1	1	0	2	1	10
<i>H. syriacus</i>	1	1	1	0	2	1	6-8
<i>H. tiliaceus</i>	1	1	1	0	2	1	7-10
<i>H. trionum</i>	1	1	1	0	2	1	12
<i>H. waimeae</i>	1	1	1	0	2	1	–
<i>Kosteletzkya virginica</i>	1	1	1	0	2	0	8-11
<i>K. pentacarpos</i>	1	1	1	0	2	0	6-11

Note: Fruit type (FT): schizocarp =0, 1= capsule; Filament tubes with anthers insert along length or at apex (FTAILA): 0 = at apex; 1= along length; Styles (ST): 0 = confluent, 1 = divided; Relationship between style branches and ovary locules (RSBOL): 0 = equal, 1 = style branches *ca.* $2 \times$ as many as carpels; Stigmata shape (SS): 0 = filiform, 1 = lobed or flat, 2 = capitate, 3 = club-shaped; Ovule number per ventricle (ONV): 0 = one ovule per ventricle, 1= two or more than ovules per ventricle; EN: epicalyx numbers; “–” indicates information is missing (unknown) or not existent.



Fig. 5 Evolution of fruit type as optimized on the *ndhF* (A) and *rpl16* (B) MP tree by MacClade.

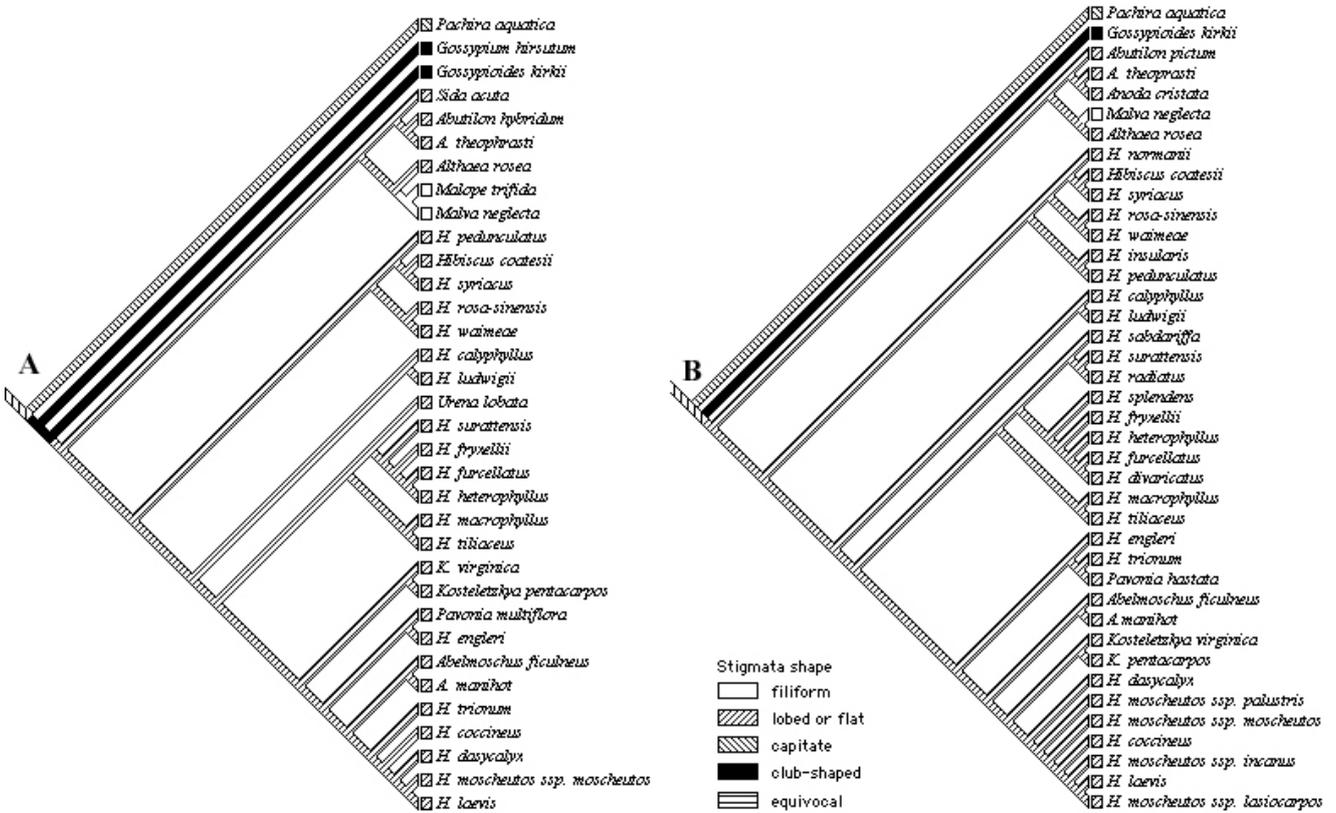


Fig. 6 Evolution of stigmata shape as optimized on the *ndhF* (A) and *rpl16* (B) MP tree by MacClade.

ma as the ancestral state, filiform, capitate and club-shaped stigma all had evolved once in the *ndhF* (Fig. 6A) or *rpl16* (Fig. 6B) tree. When we regarded two or more than ovules per ventricle as the ancestral state, one ovule per ventricle had evolved five times in the *ndhF* (Fig. 7A) and four times

in the *rpl16* (Fig. 7B) tree.

Hutchinson (1969) noted that morphological evolution within Malvaceae is centred around the development of the involucre of bracteoles. Akpan and Hossain (1998) suggested that *H. surattensis* with 10 epicalyx segments is more

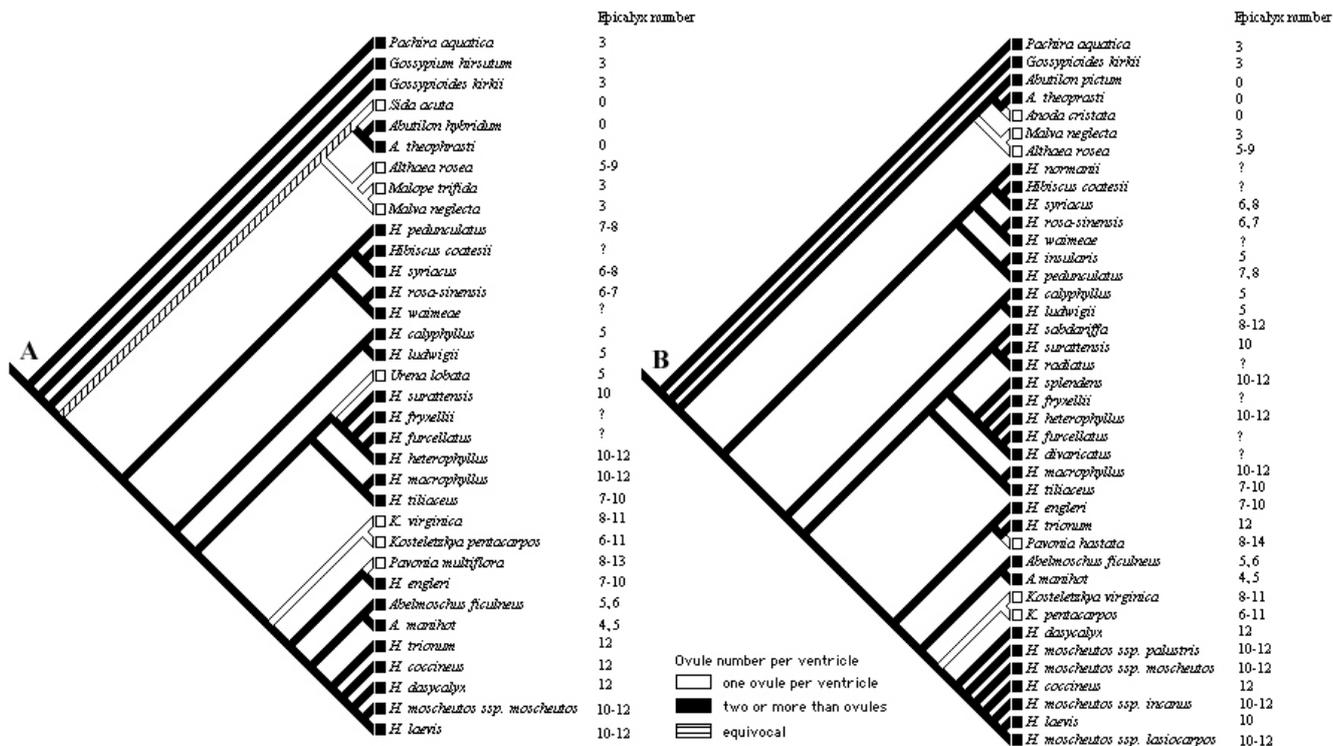


Fig. 7 Evolution of ovule number per ventricle as optimized on the *ndhF* (A) and *rpl16* (B) MP tree by MacClade.

primitive than *H. asper* with six epicalyx segments and *H. cannabinus* with nine epicalyx segments, with *H. asper* the most advanced of the three species. When the epicalyx numbers on the *ndhF* and *rpl16* MP trees were marked, we found that species with similar epicalyx number were clustered in the same or near group (Fig. 7). This indicates that it is reasonable for epicalyx number to be an important evidence of circumscribing the Malvaceae.

Evolution of style curvature and correlated traits

To investigate the evolution of style curvature, Ruan *et al.* (unpublished) mapped the trait state of styles and correlated characters on the molecular phylogenetic trees of 52 species of 13 genera of this family reconstructed using chloroplast DNA sequences of *ndhF* and the *rpl16* intron, in which 23 species belonged to eight genera present style curvature. Their results showed that (i) the no-curvature state of styles can be considered as the ancestral state for the Malvaceae; style curvature should have independently evolved seven times in the *ndhF* tree including 17 species with style curvature that belong to eight genera, eight times in the *rpl16* tree including 20 species with style curvature that belong to six genera, and five times in the *ndhF+rpl16* tree including 14 species with style curvature that belong to five genera (Ruan *et al.* unpublished); (ii) the number of transitions of HK is twice in the *ndhF* MP tree, three times in the *rpl16* MP tree, and twice in the *ndhF+rpl16* MP tree; (iii) when we regard perennial as the ancestral state, annual and annual/perennial appear to have evolved five times and twice in the *ndhF* MP tree, respectively, seven and three times in the *rpl16* MP tree, respectively, and five and three times in the *ndhF + rpl16* MP tree, respectively; (iv) if we regard trees as the ancestral state, herbs and herbs/shrubs appear to have evolved seven times and twice in the *ndhF* MP tree, respectively, seven times and twice in the *rpl16* MP tree, respectively, and four times and once in the *ndhF + rpl16* MP tree, respectively; and (v) if we regard perennial shrubs and/or trees as the ancestral state, annual and/or perennial herbs appear to have evolved seven times in the *ndhF* MP tree, seven times in the *rpl16* MP tree, and four times in the *ndhF + rpl16* MP tree; annual/perennial herbs/shrubs (*H. coatesii* and *H. pedunculatus*) have evolved twice, twice and once in the *ndhF*, *rpl16* and *ndhF + rpl16* MP trees,

respectively.

Evolutionary relationship of style curvature and plant type-habit

In the Malvaceae, the transition to use style curvature is associated with a shift to annual or perennial herbs (Ruan *et al.* unpublished). When we exclude species without style branches and HK and except for three perennial-shrub species of *Pavonia hastata*, *P. multiflora* and *Urena lobata*, style curvature and plant type-habit appear to have similar evolutionary patterns based on the molecular phylogenetic trees.

Extant natural variation for a particular trait often reflects the evolutionary history of the correlated traits (Stearns 2000). Annuals must successfully pollinate and reproduce during the year in which they open flowers. For example, the evolution of the perennial strategy from the annual strategy in the grass *Poa annua* included changes in the number and size of inflorescences and allocation of resources towards growth versus reproduction (Till-Bottraud *et al.* 1990). In the Malvaceae, species with herkogamous flowers evolved to annual or perennial herbs, commonly accompanying the selection of style curvature for maximizing reproductive success in the life history. In the *Kosteletzkya* genus of this family, style curvature occurs in the perennial herbaceous species of *K. virginica* and *K. pentacarpos*, but not in the perennial shrub or tree species of *K. diplocrater* and *K. velutina*. Even two annual and/or perennial herbaceous and/or shrub species, namely *H. coatesii* and *H. pedunculatus*, also present style curvature.

Unpredictable pollinator environments triggering the evolution of style curvature

The evolution of context-dependent style curvature may have been associated with the distribution of annual or perennial herbaceous species in an ecological niche with variable pollination conditions. Twenty-three species with style curvature in the Malvaceae are mostly distributed in unpredictable pollinator environments, including salt-marshes, wetlands, streamsides, riversides, forests and farm margins, etc. (Ruan *et al.* unpublished).

The environments that contribute most to reproduction

are thus also the environments that most strongly shape the reaction norm, and those that contribute little to reproduction have correspondingly little effect on the reaction norm (Stearns 2000). In the Malvaceae, eight species with style curvature, which are naturally distributed in North America and were clustered in the same clade of the molecular phylogenetic tree, have similar ecological affinities (all species are primarily wetland species, either in marshes or along rivers, drainage ditches, or lake edges), a common growth habit being perennial herbs that grow back from a rootstock each year (Ruan *et al.* unpublished). These indicate, for annual or perennial herbaceous species with herkogamous flowers in the Malvaceae, that unpredictable pollinator environments may promote the evolution of style curvature to achieve reproductive assurance when pollinators are scarce or absent. For example, adverse weather conditions significantly reduced pollinator visitation rates, and increased the incidence of style curvature in *K. virginica* (Ruan *et al.* 2009a).

Biogeographical history of style curvature

Species with style curvature that originate from the southern hemisphere, *H. pedunculatus* from Southeast Africa and North Australia and *H. coatesii* from Australia were grouped together in the *ndhF* tree, but *H. surattensis* from Africa and Australia was in a different clade from that of *H. pedunculatus* (Ruan *et al.* unpublished). In the *rpl16* and *ndhF + rpl16* trees constructed by Ruan *et al.* (unpublished), two species, *Pavonia hastata* and *P. multiflora* from South America, were clustered in a different clade from that of *H. coatesii* and *H. normanii*, which are naturally distributed in Australia, but were grouped into a clade including *A. ficulneus* that is distributed in Northern Australia. These inconsistencies indicate that the circumscriptions of the biogeographical history of style curvature, occurring in species originating from the Southern hemisphere, will not have been unique.

Eight of 23 species with style curvature sharing a North American geographic distribution and common ecological distributions were grouped into the same clade (Ruan *et al.* unpublished). In the *Kosteletzkya* genus, *K. pentacarpos* and *K. virginica*, which are naturally distributed in Europe and North America, respectively and grouped in the same clade, share a common ecological trait (style curvature) and environmental conditions (wetlands along the seaside). This is consistent with Crisp *et al.*'s suggestions (2009) that biome conservatism at speciation, in which species retain ancestral ecological traits and environmental distributions, has outweighed biome shifts and was also prevalent in transoceanic dispersals.

ADAPTIVE SIGNIFICANCE OF STYLE CURVATURE

Adaptive significance

Since Darwin (1862) noted that reconfiguration in orchid pollinia avoids self-pollination (Peter and Johnson 2006), various hypotheses have been proposed to explain the adaptive significance of diversely floral movements. These include reduction in intrafloral male-female interference (Yang *et al.* 2004; Bai and Zhang 2005; Edwards *et al.* 2005; Sun *et al.* 2007), avoidance of self-pollination and promotion of outcrossing (Schlessman 1986; Li *et al.* 2001; Verma *et al.* 2004; Peter and Johnson 2006), delayed autonomous pollination (Wang *et al.* 2004; Ruan *et al.* 2004; Liu *et al.* 2006) and tolerance of harsh environments (Bynum and Smith 2001; Huang *et al.* 2002). However, adaptive significance of floral movement in some species has yet to be fully explained, presenting some challenging problems for evolutionary biologists. For example, Li *et al.* (2001) believed that flexistylis in *Alpinia kwangsiensis* evolved in flowering plants to encourage outcrossing (Li *et al.* 2002) but Sun *et al.* (2007) showed that flexistylis in *A. blephar-*

calyx most likely represents a means to reduce interference with pollen export. Moreover, it is very difficult to distinguish the reduction in intrafloral male-female interference from the avoidance of self-pollination. Additional studies also corroborated that a single floral movement could have more than one adaptive mechanism (Buttrose *et al.* 1977; Schlessman 1986). For example, stylar movement in *Eremurus himalaicus* avoids self-pollination and promotes outcross-pollination (Verma *et al.* 2004), which also ensures that the style is separated/displaced from the flight path of visitors. This may avoid the influence of the pistil on pollen export and improve male fitness.

Style curvature in the Malvaceae has been interpreted as a mechanism to achieve delayed selfing and/or the promotion of outcrossing. Further more, Ruan *et al.* (2010) presume that style curvature may also serve an alternative function, namely to reduce intrafloral male-female interference.

Promotion of outcrossing

First, style curvature positions stigmas in the path of the visiting pollinator and takes a very long time (5-9 h for *K. virginica*) before bringing stigmas into contact with their own anthers, which offers more opportunity to receive outcrossing pollen. Stephens (1948) interpreted style curvature of *H. laevis* as a mechanism to foster outcrossing by positioning the stigmas in the flight path of nectar-seeking bees. Similarly, this functional significance should be reflected in other species with Type I curvature, such as *H. pedunculatus*, *H. moscheutos* and *K. pentacarpos*. On the other hand, the adaptive mechanism of promoting outcrossing is also evidenced by Type II curvature in *A. rosea*, *M. trifida* and *M. neglecta*. In these three species, when style branches exert out of the monadelphous column and begin to curve, most pollen is shed and pollen viability is lower when the curvature brings stigmas near to or in contact with the anthers, but curved process is favor of contact between stigmas and visitors. Floral movement encouraging outcrossing has also been reported in *Ourisia macrocarpa* (Scrophulariaceae) (Schlessman 1986), *A. kwangsiensis* (Zingiberaceae) (Li *et al.* 2001), *E. himalaicus* (Liliaceae) (Verma *et al.* 2004) and *Eulophia parviflora* (Orchidaceae) (Peter and Johnson 2006).

Delayed selfing pollination

In the process of long-time style curvature, if pollination fails, the curvature finally results in delayed self-pollination. Blanchard (1976) regarded the style movement of *H. trionum* as a means to achieve selfing, citing observations of stigmas eventually recurring into the anther mass. This delayed selfing via un-pollinated style curvature occurred in *H. lavis* (Klips and Snow 1997) and *K. virginica* (Ruan *et al.* 2004) and is likely to exist in the species of the Malvaceae with Type I curvature. Floral movements that achieve delayed selfing have also been reported in *Aquilegia canadensis* (Ranunculaceae) (Herlihy and Eckert 2002), *Collinsia verna* (Scrophulariaceae) (Kalisz *et al.* 2004), *Caulokaempferia coenobialis* (Zingiberaceae) (Wang *et al.* 2004) and *Holcoglossum amesianum* (Orchidaceae) (Liu *et al.* 2006).

Reduction in intrafloral male-female interference

The decrease in stigma-anther separation via style curvature may reduce intrafloral male-female interference from too great approach HK, and maximize mating opportunity offered by style culture also improves male-female fitness. For *K. virginica* with a large corolla (diameter = 53.11 ± 0.83 mm) and approach HK of 3.64 ± 0.08 mm, the length of the body of most pollinators is less than that of the monadelphous column (19.77 ± 0.38 mm) and that of the style (23.40 ± 0.37 mm). For example, the body length (about 22.7 mm) of only *Bombus speciosus* of 12 categories

of pollinators visiting *K. virginica* flowers in the Jiansu populations of China is more than that of the monadelphous column. Most pollinators collecting pollen or honey directly visit the flower through the flank of its corolla, which results in pollinators apart from stigmas and only having contact with the stamens when styles do not curve. This distance interference between male-female functions may belong to stamens that prevent stigmas from just contacting with pollinators (Bertin and Newman 1993; Barrett 2002), resulting in the decrease of male-female fitness. Hence, the decrease of approach HK from style curvature avoids to a certain degree the above too-great-a-distance interference, because style curvature that positions the stigmas in the flight path of visiting pollinators favors receiving self- and outcrossing-pollen, which improves male-female fitness.

Fetscher's (2001) study provides the first experimental evidence that selection to reduce intrafloral male-female interference can be a strong selective force and can drive the evolution of floral characters usually interpreted as mechanisms to reduce self-fertilization. At present, increasing evidence also supports that some floral movement may act as a mechanism to reduce interference between the reproductive functions of male and female organs (Bennington 2003; Yang *et al.* 2004; Edwards *et al.* 2005; Sun *et al.* 2007). In the male floral morph of *Passiflora incarnata*, the pistil is similar in size to that of hermaphrodite flowers, but the styles are held erect where they are unlikely to come into contact with pollinators. However, hermaphrodite flowers deflect their styles to a position where contact with pollen-carrying pollinators is likely (Bennington 2003), improving pollen reception. Yang *et al.* (2004) showed that the stigma behavior in *Campsis radicans* is not an outcrossing mechanism, but strongly indicates that it acts as a mechanism to facilitate pollination, thus enhancing reproductive success.

We in general recognize that one floral movement in one species has one or two functional mechanisms, whereas the presumption of three adaptive significances will be a challenging notion for evolutionary biologists. However, Ruan *et al.* (2010) conclude that the curvature of unpollinated styles in *K. virginica* is simultaneously involved in three functions, including the reduction in intrafloral male-female interference from too great approach HK (Ruan *et al.* 2005b), the promotion of outcrossing and the achievement of delayed selfing (Ruan *et al.* 2004). In *K. virginica*, if pollination fails, unpollinated styles curve and position the stigmas in the flight path of visiting pollinators or provide the precondition for self-pollination combining style curvature and corolla closure (Ruan *et al.* 2005a, 2005b), which favors the receipt of self- and outcrossing-pollen, to a certain degree reducing the above interference between intrafloral male-female functions from too great approach HK (first adaptive significance). In the first half of the process of style curvature, the upper surface of stigmas, which is very receptive, is located in the path of pollinators visiting the flower, which will increase the veracity of cross-pollen transfer (Stephens 1948) (second adaptive significance). When pollinators retreat from the flowers, the lower surface of stigmas has no receptive contact with pollinators, which avoids self-pollination. However, if pollination always fails, the curvature will continue to bring stigmas into contact with their own anthers, potentially resulting in delayed selfing (Ruan *et al.* 2004) (third adaptive significance).

Reproductive assurance

Delayed selfing is always advantageous because it occurs after the opportunity for outcross pollen receipt has passed. It assures seed production when pollinators are scarce (reproductive assurance) while allowing outcrossing to dominate when they are abundant (Klips and Snow 1997; Kalisz *et al.* 2004). However, although delayed selfing has been reported in many plants, there are only a few cases in which its impact on reproductive assurance has been tested quantitatively (Eckert and Schaefer 1998; Donnelly *et al.* 1998;

Nagy *et al.* 1999; Herlihy and Eckert 2002; Kalisz and Vogler 2003; Kalisz *et al.* 2004). In some cases, delayed autonomous selfing has been reported to increase reproductive success. The amount of autonomous selfing by *Collinsia verna* varies depending on pollinator conditions (Kalisz and Vogler 2003), with the amount of selfing increasing when decreasing pollinators abundance; and delayed selfing in *C. verna* provides reproductive assurance (Kalisz *et al.* 2004). Emasculated small flowers produce very few seeds, relative to intact small flowers in *C. parviflora* (Elle and Carney 2003). Under natural pollination, emasculated flowers in *Verbascum thapsus* without delayed selfing experience a reduction in seed set relative to intact flowers with delayed selfing (Donnelly *et al.* 1998). In contrast, other available studies indicate that autonomous selfing does not substantially increase seed set. By comparing intact with emasculated open-pollinated flowers, Nagy *et al.* (1999) found that autonomous self-pollination in *Kalmia latifolia* does not increase fruit production. In *Aquilegia canadensis*, Herlihy and Eckert (2002) found that autonomous selfing increases seed production; but that this benefit is outweighed by the loss of high quality seed as a result of seed discounting and inbreeding depression.

In species of the Malvaceae with delayed selfing, Ruan *et al.* (2009b) founded that the mean number of seeds per capsule from intact open-pollinated flowers was higher than that of emasculated open-pollinated flowers in *K. virginica*. We also discovered that adverse weather conditions significantly increased the incidence of delayed selfing (Ruan *et al.* 2005b, 2009a). Furthermore, elimination of delayed selfing by experimental manipulation reduced fruit and seed set compared to non-emasculated flowers, even under adverse weather conditions (Ruan *et al.* 2009c). These indicated that *K. virginica* had a well-developed capacity for delayed autonomous selfing, which provide reproductive assurance. However, Klips and Snow (1997) observed that autonomous self-pollination in *H. laevis* did not contribute significantly to fruit production of open pollinated flowers.

Mixed mating system

In the Malvaceae, the species with delayed selfing via style curvature are mostly like to represent a mixed mating system. This because (i) the occurrence of style curve responds to pollination environments; and (ii) curved process promotes outcrossing and curve end achieves delayed selfing. In the same two naturalized populations of *K. virginica*, results based on AFLP markers indicated that mean inbreeding depression was 0.640 ± 0.006 , pollinator failure rates at the two naturalized *K. virginica* populations during 2005-2007 ranged from 0.091 ± 0.039 to 0.174 ± 0.061 , and selfing rate ranged from 0.247 ± 0.018 to 0.468 ± 0.031 ; population selfing rates are significantly and positively correlated with field estimates of pollinator failure ($P = 0.008 < 0.01$, $R^2 = 0.857$) (Ruan *et al.* 2009b). This provides an empirical demonstration of a mixed mating system response to the pollinator environment in the presence of strong inbreeding depression.

Despite selection against self-pollination, mixed mating is frequent in seed plants (Schoen and Brown 1991; Bertin and Newman 1993; Barrett *et al.* 1996; Johnston 1998; Vogler and Kalisz 2001; Zhang 2004; Goodwillie *et al.* 2005); and the transition from outcrossing to self-fertilization is one of the most common evolutionary trends in plants (Stebbins 1957, 1974; Holsinger 2000; Barrett 2002; Herlihy and Eckert 2002). Unpredictable pollination environments are the norm in wild plant populations, and can include periods of total pollinator failure (Cruden and Lyon 1989; Burd 1994). If the availability of outcross pollen is inconsistent within or between years, such conditions would favor self-pollination (Schoen and Brown 1991), resulting in a mixture of self and outcross seed production (mixed mating). Species with floral developmental mechanisms that promote outcrossing when pollinators are present, but ensure self-pollination if they are not (Lloyd 1992; Kalisz

and Vogler 2003), can have different annual selfing rates as a functional response to pollinator environments, assuring reproduction and providing a 'best of both worlds' mating system (Cruden and Lyon 1989). Since Darwin (1876) and Fisher (1941), questions surrounding the evolution of self-fertilization and the maintenance of mixed mating systems have remained at the forefront of evolutionary biology (Goodwillie *et al.* 2005). Darwin (1876) proposed that an adaptive benefit of self-pollination is to provide reproductive assurance when the opportunity of outcrossing has passed (Baker 1955; Lloyd 1992; Kalisz *et al.* 2004). Self-pollination under variable pollinator environments can be advantageous (Kalisz and Vogler 2003; Kalisz *et al.* 2004), because survival of any progeny produced by delayed selfing provides reproductive assurance (Schoen and Brown 1991). A comparative analysis of estimated inbreeding coefficients and outcrossing rates suggests that mixed mating often evolves despite strong inbreeding depression (Goodwillie *et al.* 2005), but there is few experimental tests of this perspective.

CONCLUSION

The controversial circumscription of the Malvaceae based on un-uniquely morphological features within the family or among the families of Malvales has long intrigued evolutionary biologists. Although increasing molecular phylogenetic studies have greatly improved our understanding of Malvaceae systematics and results in the recircumscription of major taxonomic groups.

Hermaphroditic flowers have features to promote outcrossing, including showy petals to attract pollinators, pollen and nectar rewards to pollinator, and approach HK from monadelphous stamen; however, when pollination fails, unpollinated styles curve and bring stigmas to contact the anthers, possibly resulting delayed selfing. This curvature occurs in 23 species distributed in eight genera of four tribes (Malvaceae, Urenaee, Hibisceae, Malveae), and has evolved at least eight times. A shift to use style curvature is associated with a shift to annual or perennial herbs, and an unpredictable pollinator environment is likely an important trigger for this evolution. The adaptive significance of style curvature in the Malvaceae includes delayed selfing, promotion of outcrossing and reduction in intrafloral male-female interference, even if the frequency of two or three of these occurs in only one species. This diversity provides an opportunity for future studies of floral movement, including the evolution, relative importance and harmony mechanism of different adaptive significances.

FUTURE RESEARCH

Context-dependent style curvature promotes delayed selfing and provides reproductive assurance in the absence of pollinators, representing mixed mating system; however, how to evolve and evolutionary relationship between them are still an enigma. In the face of this interesting challenge, in future research, we should simultaneously document broader species in the Malvaceae to determine in which styles curve or not and the plant traits (e.g., reproductive character, nutritive character, micromorphological and embryonic character, breeding system, pollination biology and breeding system) to test the relationship between style curvature and plant traits. Such an important study would result in the construction of a completely new phylogenetic tree of the Malvaceae and would allow the mapping of tree characters to be conducted to determine the evolution of style curvature and its co-evolution with other plant traits. In addition, more studies should be (1) done about relative importance and harmony mechanism of different adaptive significance of this dramatic floral mechanism; and (2) carried out on the pollination and mating systems of the Malvaceae in a wider range, and phylogenetic research should be conducted to understand the evolution of pollination and mating system in Malvaceae.

ACKNOWLEDGEMENTS

The author thanks Dr. Jaime A. Teixeira da Silva for his suggestions and revision of this manuscript. Funding for this work was provided by the National Natural Science Foundation of China (Grant no. 30500071 to C.-J. Ruan).

REFERENCES

* in Chinese with English abstract

- Akpan GA (2000) Cytogenetic characteristics and the breeding system in six hibiscus species. *Theoretical and Applied Genetics* **100**, 315-318
- Akpan GA, Hossain MG (1998) Karyotypes and evolutionary relations of *Hibiscus asper* Hook., *H. camabinus* L. and *H. surattensis* L. (Malvaceae). *Botanical Journal of the Linnean Society* **126**, 207-216
- Alverson WS, Karol KG, Baum DA, Chase MW, Swensen SM, McCourt R, Sytsma KJ (1998) Circumscription of the Malvales and relationships to other Rosidae: Evidence from RBCL sequence data. *American Journal of Botany* **85**, 867-887
- Alverson WS, Whitlock BA, Nyffeler R, Bayer C, Baum DA (1999) Phylogeny of the core Malvales: evidence from *ndhF* sequence data. *American Journal of Botany* **86**, 1474-1486
- Bai WN, Zhang DY (2005) Sexual interference in cosexual plants and its evolutionary implications. *Journal of Plant Ecology* **29**, 672-679*
- Baker HG (1955) Self-compatibility and establishment after 'long-distance' dispersal. *Evolution* **9**, 347-348
- Barrett SCH (2002) The evolution of plant sexual diversity. *Nature Reviews Genetics* **3**, 274-284
- Barrett SCH (2003) Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **358**, 991-1004
- Barrett SCH, Harder LD, Worley AC (1996) The comparative biology of pollination and mating in flowering plants. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **351**, 1271-1280
- Bates DM (1968) Generic relationships in the Malvaceae, tribe Malveae. *Gentes Herbarum* **10**, 117-135
- Baum DA, Small RL, Wendel JF (1998) Biogeography and floral evolution of Baobabs (*Adansonia*, Bombacaceae) as inferred from multiple data sets. *Systematic Biology* **47**, 181-207
- Baum DA, Smith SD, Yen A, Alverson WS, Nyffeler R, Whitlock BA, Oldham RL (2004) Phylogenetic relationships of Malvaceae (Bombacoideae and Malvoideae; Malvaceae *sensu lato*) as inferred from plastid DNA sequences. *American Journal of Botany* **91**, 1863-1871
- Bayer C, Fay MF, De Bruijn AY, Savolainen V, Morton CM, Kubitzki K, Alverson WS, Chase MW (1999) Support for an expanded family concept of Malvaceae with a recircumscribed order Malvales: a combined analysis of plastid *atpB* and *rbcL* DNA sequences. *Botanical Journal of the Linnean Society* **129**, 267-303
- Belaoussoff S, Shore JS (1995) Floral correlates and fitness consequences of mating-system variation in *Turnera ulmifolia*. *Evolution* **49**, 545-556
- Bennington C (2003) Sex allocation and stylar movement in *Passiflora incarnata*. <http://abstracts.co.allenpress.com/pweb/esa2003/document/?ID=26171>
- Bertin RI, Newman CM (1993) Dichogamy in angiosperms. *Botanical Review* **59**, 112-152
- Blanchard OJ (1976) A revision of species segregated from *Hibiscus* sect. *Triomum* (Medicus) de *Candolle sensu lato* (Malvaceae). PhD thesis, Cornell University, USA, pp 21-93
- Brunet J, Eckert CG (1998) Effects of floral morphology and display on outcrossing in blue columbine, *Aquilegia caerulea* (Ranunculaceae). *Functional Ecology* **12**, 596-606
- Burd M (1994) Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. *Botanical Review* **60**, 83-139
- Buttrose MS, Grant WJR, Lott JNA (1977) Reversible curvature of style branches of *Hibiscus trionum* L., a pollination mechanism. *Australian Journal of Botany* **25**, 567-570
- Bynum MR, Smith WK (2001) Floral movements in response to thunderstorms improve reproductive effort in the alpine species *Gentiana algida* (Gentianaceae). *American Journal of Botany* **88**, 1088-1095
- Cheptou P-O, Schoen DJ (2007) Combining population genetics and demographical approaches in evolutionary studies of plant mating systems. *Oikos* **116**, 271-279
- COSEWIC (2004) COSEWIC assessment and update status report on the swamp rose-mallow *Hibiscus moscheutos* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. Vii+43 pp. Available online: www.sararegistry.gc.ca/status/status_e.cfm
- Craven LA, Wilson FD, Fryxell PA (2003) A taxonomic review of *Hibiscus* section *Furcaria* (Malvaceae) in Western Australia and the Northern Territory. *Australian Journal of Botany* **16**, 185-218
- Crisp MD, Arroyo MTK, Cook LG, Gandolfo MA, Jordan GJ, McGlone MS, Weston PH, Westoby M, Wilf P, Linder HP (2009) Phylogenetic biome conservatism on a global scale. *Nature* **458**, 754-756

- Cruden RW, Lyon DL** (1989) Facultative xenogamy: examination of a mixed mating system. In: Bock JH, Linhart YB (Eds) *The Evolutionary Ecology of Plants*, Westview, Boulder, Colorado, pp 171-207
- Darwin C** (1862) *On the Various Contrivances by which British and Foreign Orchids are Fertilized by Insects*, John Murray Press, London, pp 163-242
- Darwin C** (1876) *The Effects of Cross and Self-fertilization in Vegetable Kingdom*, John Murray Press, London, pp 63-75
- Dole JA** (1990) Role of corolla abscission in delayed self-pollination of *Mimulus guttatus* (Scrophulariaceae). *American Journal of Botany* **77**, 1505-1507
- Donnelly SE, Lortie CJ, Aarssen LW** (1998) Pollination in *Verbascum thapsus* (Scrophulariaceae): the advantage of being tall. *American Journal of Botany* **85**, 1618-1625
- Eckert CG, Schaefer A** (1998) Does self-pollination provide reproductive assurance in *Aquilegia canadensis* (Ranunculaceae)? *American Journal of Botany* **85**, 919-924
- Edlin HL** (1935) A critical revision of certain taxonomic groups of the Malvaceae. *New Phytologist* **34**, 122-124
- Edwards J, Whitaker D, Klionsky S, Laskowski MJ** (2005) A record-breaking pollen catapult. *Nature* **435**, 164
- Elle E, Carney R** (2003) Reproductive assurance varies with flower size in *Collinsia parviflora* (Scrophulariaceae). *American Journal of Botany* **90**, 888-896
- Elle E, Hare JD** (2002) Environmentally induced variation in floral traits affects the mating system in *Datura wrightii*. *Functional Ecology* **16**, 79-88
- Faegri K, van der Pijl L** (1979) *The Principles of Pollination Ecology*, Pergamon, Oxford, pp. 13-21
- Feng KM** (1984) *Flora* (Vol 49 (2)), Science Press, Beijing, pp 1-238*
- Fernald ML** (1942) *Hibiscus moscheutos* and *H. palustris*. *Rhodora* **44**, 266-278
- Fetscher AE** (2001) Resolution of male-female conflict in a hermaphroditic flower. *Proceedings of the Royal Society of London Series B: Biological Sciences* **268**, 525-529
- Fetscher AE, Kohn JR** (1999) Stigma behavior in *Mimulus aurantiacus* (Scrophulariaceae). *American Journal of Botany* **86**, 1130-1135
- Fisher RA** (1941) Average excess and average effect of a gene substitution. *Annals of Eugenics* **11**, 53-63
- Fryxell PA** (1968) A redefinition of the tribe Gossypieae. *Botanical Gazette* **129**, 296-308
- Fryxell PA** (1975) Generic relationships of *Decaschistia* (Malvaceae) and the description of a new tribe, Decaschistieae. *American Journal of Botany* **62**, 172-175
- Fryxell PA** (1988) Malvaceae of Mexico. *Systematic Botany Monographs* **25**, 1-522
- Goodwillie C, Kalisz S, Eckert CG** (2005) The evolutionary enigma of mixed mating systems in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology and Systematics* **36**, 47-79
- Herlihy CR, Eckert CG** (2002) Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* **416**, 320-323
- Holsinger KE** (2000) Reproductive systems and evolution in vascular plants. *Proceedings of the National Academy of Sciences USA* **97**, 7037-7042
- Holtsford TP, Ellstrand NC** (1992) Genetic and environmental variation in floral traits affecting outcrossing rate in *Clarkia tembloriensis* (Onagraceae). *Evolution* **46**, 216-225
- Huang SQ, Takahashi Y, Dafni A** (2002) Why does the flower stalk of *Pulsatilla cernua* (Ranunculaceae) bend during anthesis? *American Journal of Botany* **89**, 1599-1603
- Hutchinson J** (1967) *The Genera of Flowering Plants (Angiospermae) Dicotyledons* (Vol II), Clarendon Press, London, pp 63-92
- Hutchinson J** (1969) *Evolution and Phylogeny of Flowering Plants. Dicotyledons: Facts and Theory*, Academic Press, London, pp 75-86
- Jesson LK, Barrett SCH** (2002) Solving the puzzle of mirror-image flowers. *Nature* **417**, 707
- Johnston MO** (1998) Evolution of intermediate selfing rates in plants: pollination ecology versus deleterious mutations. *Genetica* **102/103**, 267-278
- Judd WS, Manchester SR** (1997) Circumscription of Malvales (Malvaceae) as determined by a preliminary cladistic analysis of morphological, anatomical, palynological and chemical characters. *Brittonia* **49**, 384-405
- Juncosa AM, Webster BD** (1989) Pollination in *Lupinus nanus* (Leguminosae). *American Journal of Botany* **76**, 59-66
- Kalisz S, Vogler D, Fails B, Finer M, Shepard E, Herman T, Gonzales R** (1999) The mechanism of delayed selfing in *Collinsia verna* (Scrophulariaceae). *American Journal of Botany* **86**, 1239-1247
- Kalisz S, Vogler DW** (2003) Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* **84**, 2928-294
- Kalisz S, Vogler DW, Hanley KM** (2004) Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* **430**, 884-887
- Karron JD, Jackson RT, Thumser NN, Schlicht SL** (1997) Outcrossing rates of individual *Mimulus ringens* genets are correlated with anther-stigma separation. *Heredity* **79**, 365-370
- Kennedy H** (1999) Explosive secondary pollen presentation in family Marantaceae. Botanical Electronic News. Available online: <http://www.ou.edu/cas/botany-micro/ben/ben216.html>
- Klips RA, Snow AA** (1997) Delayed autonomous self-pollination in *Hibiscus laevis* (Malvaceae). *American Journal of Botany* **84**, 48-53
- Koopman MM, Baum DA** (2008) Phylogeny and biogeography of tribe Hibisceae (Malvaceae) on Madagascar. *Systematic Botany* **33**, 364-374
- Kudoh H, Wigham** (1998) The effect of petal size manipulation on pollinator/seed-predator mediated female reproductive success of *Hibiscus moscheutos*. *Oecologia* **117**, 70-79
- Li QJ, Kress WJ, Xu ZF, Xia Y, Zhang L, Deng XB, Gao J** (2002) Mating system and stigmatic behaviour during flowering of *Alpinia kwangsiensis* (Zingiberaceae). *Plant Systematics and Evolution* **232**, 123-132
- Li QJ, Xu ZF, Kress WJ, Xia Y, Zhang L, Deng XB, Gao J, Bai Z** (2001) Flexible style that encourages outcrossing. *Nature* **410**, 432
- Liu KW, Liu ZJ, Huang LQ, Li LQ, Chen LJ, Tang GD** (2006) Self-fertilization strategy in an orchid. *Nature* **441**, 945-946
- Lloyd DG** (1992) Self- and cross- fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Sciences* **153**, 370-380
- Lloyd DG, Schoen DJ** (1992) Self- and cross-fertilization in plants. I Functional dimensions. *International Journal of Plant Sciences* **153**, 358-369
- Lyon DL** (1992) Bee pollination of facultatively xenogamous *Sanguinaria canadensis* L. *Bulletin of the Torrey Botanical Club* **119**, 368-375
- McDade LA, Davidar P** (1984) Determinants of fruit and seed set in *Pavonia dasypetala* (Malvaceae). *Oecologia* **64**, 61-67
- Miyajima D** (2001) Floral variation and its effect on self-pollination in *Salvia splendens*. *Journal of Horticultural Science and Biotechnology* **76**, 187-194
- Motten AF, Stone JL** (2000) Heritability of stigma position and the effect of stigma-anther separation on outcrossing in a predominantly self-fertilizing weed, *Datura stramonium* (Solanaceae). *American Journal of Botany* **87**, 339-347
- Nagy ES, Strong L, Galloway LF** (1999) Contribution of delayed autonomous selfing to reproductive success in Mountain Laurel, *Kalmia latifolia* (Ericaceae). *American Midland Naturalist* **142**, 39-46
- Peter CI, Johnson SD** (2006) Doing the twist: a test of Darwin's cross-pollination hypothesis for pollinium reconfiguration. *Biology Letters* **2**, 65-68
- Pfeil BE, Brubaker CL, Craven LA, Crisp MD** (2002) Phylogeny of *Hibiscus* and the tribe Hibisceae (Malvaceae) using chloroplast DNA sequences of *ndhF* and the *rpl16* intron. *Systematic Botany* **27**, 333-350
- Ramsey M, Seed L, Vaughton G** (2003) Delayed selfing and low levels of inbreeding depression in *Hibiscus trionum* (Malvaceae). *Australian Journal of Botany* **51**, 275-281
- Ratheke B, Real L** (1993) Autogamy and inbreeding depression in mountain laurel, *Kalmia latifolia* (Ericaceae). *American Journal of Botany* **80**, 143-146
- Ratheke BJ** (2000) Birds, pollination reliability, and green flowers an endemic island shrub, *Pavonia bahamensis* (Malvaceae). *Rhodora* **102**, 292-414
- Ruan CJ** (2007) Role of corolla closure in delayed self-pollination of *Kosteletzkya virginica* (Malvaceae). *Research and Reviews in BioSciences* **1**, 34-37
- Ruan CJ, Jiang GB** (2007) Adaptive significance of herkogamy and floral behaviour. *Journal of Plant Ecology* **30**, 210-220*
- Ruan CJ, Li H, Mopper S** (2009b) *Kosteletzkya virginica* displays mixed mating system responding to pollinator environment despite strong inbreeding depression. *Plant Ecology* **203**, 183-193
- Ruan CJ, Mopper S, Teixeira da Silva JA, Qin P, Shan Y** (2009a) Context-dependent style curvature within flowers offers reproductive assurance under unpredictable pollinator environments. *Plant Systematics and Evolution* **277**, 207-215
- Ruan CJ, Qin P, Han RM** (2005a) Strategies of delayed self-pollination in *Kosteletzkya virginica*. *Chinese Science Bulletin* **50**, 94-96
- Ruan CJ, Qin P, Han RM** (2005c) Floral morphology and stigma-anther separation in gynomonocious-gynodioecious *Kosteletzkya virginica* (Malvaceae). *South African Journal of Botany* **71**, 367-373
- Ruan CJ, Qin P, He ZX** (2004) Delayed autonomous selfing in *Kosteletzkya virginica* (Malvaceae). *South African Journal of Botany* **70**, 640-645
- Ruan CJ, Qin P, Teixeira da Silva JA, Zhang QX** (2009c) An experimental evaluation on function of movement approach herkogamy in *Kosteletzkya virginica* (Malvaceae). *Acta Ecologica Sinica* **29**, 98-103
- Ruan CJ, Qin P, Xi YG** (2005b) Floral traits and pollination modes in *Kosteletzkya virginica* (Malvaceae). *Belgian Journal of Botany* **138**, 39-46
- Ruan CJ, Teixeira da Silva JA, Qin P** (2010) Style curvature and its adaptive significance in the Malvaceae. *Plant Systematics and Evolution* **288**, 13-23
- Ruan CJ, Zhou LJ, Zeng FY, Han RM, Qin P, Lutts S, Saad L, Mahy G** (2008) Contribution of delayed autonomous selfing to reproductive success in *Kosteletzkya virginica* (Malvaceae). *Belgian Journal of Botany* **141**, 3-13
- Schlessman MA** (1986) Floral protogyny, self-compatibility and the pollination of *Ourisia macrocarpa* (Scrophulariaceae). *New Zealand Journal of Botany* **24**, 651-656
- Schoen DJ, Brown AHD** (1991) Whole- and part-flower self-pollination in *Glycine clandestine* and *G. argyrea* and the evolution of autogamy. *Evolution* **45**, 1665-1674
- Seed L, Vaughton G, Ramsey M** (2006) Delayed autonomous selfing and inbreeding depression in the Australian annual *Hibiscus trionum* var. *vesicarius* (Malvaceae). *Australian Journal of Botany* **54**, 27-34
- Seelanan T, Schnabel A, Wendel JF** (1997) Congruence and consensus in the cotton tribe (Malvaceae). *Systematic Botany* **22**, 259-290
- Small RL** (2004) Phylogeny of *Hibiscus* sect. *Muenchhusia* (Malvaceae) based on chloroplast *rpl16* and *ndhF*, and nuclear *ITS* and *GBSSI* sequences. *Sys-*

- tematic Botany* **29**, 385-392
- Snow AA, Spira TP** (1993) Individual variation in the vigor of self pollen and selfed progeny in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany* **80**, 160-164
- Snow AA, Spira TP, Simpson R, Klips RA** (1996) The ecology of geitonogamous pollination. In: Lloyd DG, Barrett SCH (Eds) *Floral Biology*, Chapman and Hall, New York, USA, pp 191-216
- Spira T, Snow AA, Whigham DF, Leak JL** (1992) Flower visitation, pollen deposition, and pollen-tube competition in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany* **79**, 428-433
- Spira TP** (1989) Reproductive biology of *Hibiscus moscheutos* (Malvaceae). In: Bock J, Linhart Y (Eds) *The Evolutionary Ecology of Plants*, Westview Press, Boulder, CO, pp 247-255
- Stearns SC** (2000) Life history evolution: successes, limitations, and prospects. *Naturwissenschaften* **87**, 476-486
- Stebbins GL** (1974) *Flowering Plants: Evolution above the Species Level*, Belknap, Cambridge, MA, pp 83-87
- Stebbins LG** (1957) Self fertilization and population variability in higher plants. *American Naturalist* **91**, 337-354
- Stephens WC** (1948) *Kansas Wild Flowers*, University of Kansas Press, Lawrence, Kansas, pp 69-72
- Stone JL, Motten AF** (2002) Anther-stigma separation is associated with inbreeding depression in *Datura stramonium*, a predominantly self-fertilizing annual. *Evolution* **56**, 2187-2195
- Sun S, Gao JY, Liao WJ, Li QJ, Zhang DY** (2007) Adaptive significance of flexistylis in *Alpinia blepharocalyx* (Zingiberaceae): a hand-pollination experiments. *Annals of Botany* **99**, 661-660
- Takhtajan AL** (1997) *Diversity and Classification of Flowering Plants*, Columbia University Press, New York, pp 321-478
- Tang Y, Gilbert MG, Dorr LJ** (2007) *Floral of China* (Vol 12), Science Press and Missouri Botanical Garden Press, Beijing, pp 264-298
- Thompson JD, Barrett SCH, Baker AM** (2003) Frequency-dependent variation in reproductive success in *Narcissus*: implications for the maintenance of stigma-height dimorphism. *Proceedings of the Royal Society of London, Series B, Biological Sciences* **270**, 949-953
- Till-Bottraud I, Wu L, Harding J** (1990) Rapid evolution of life history traits in populations of *Poa annua* L. *Journal of Evolutionary Biology* **3**, 205-224
- Traveset A, Willson MF, Sabag C** (1998) Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra del Fuego: a disrupted mutualism. *Functional Ecology* **12**, 459-464
- Verma S, Magotra R, Koul AK** (2004) Stylar movement avoids self-pollination and promotes cross-pollination in *Eremurus himalaicus*. *Current Science* **87**, 872-873
- Vogler DW, Kalisz S** (2001) Sex among the flowers: the distribution of plant mating systems. *Evolution* **55**, 202-204
- Wang Y, Zhang D, Renner SS, Chen Z** (2004) A new self-pollination mechanism. *Nature* **431**, 39-40
- Webb CJ, Lloyd DG** (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. II. Herkogamy. *New Zealand Journal of Botany* **24**, 163-178
- Weiss HB, Dickerson EL** (1919) Insects of the swamp rose-mallow, *Hibiscus moscheutos* L. in New Jersey. *Journal of the New York Entomological Society* **27**, 39-68
- Wu ZY, Lu AM, Tang YC** (2004) *The Families and Genera of Angiosperms in China. A Comprehensive Analysis*, Science Press, Beijing, pp 546-551*
- Yang SX, Yang CF, Zhang T, Wang QF** (2004) A mechanism facilitates pollination due to stigma behavior in *Campsis radicans* (Bignoniaceae). *Acta Botanica Sinica* **46**, 1071-1074
- Zeng FY, Zhou LJ, Ruan CJ** (2008) Comparative study on floral traits and breeding system of *Hibiscus syriacus* and *H. trionum*. *Guihaia* **28**, 750-754*
- Zhang DY** (2004) *Plant Life-History Evolution and Reproductive Ecology*, Science Press, Beijing, pp 162-173*

APPENDIX I: GLOSSARY OF TERMS

Approach herkogamy: stigmas are located above anthers and contact pollinators first upon their entry into flowers.

Biogeography: biogeography is the study of the distribution of biodiversity over space and time. It aims to reveal where organisms live, and at what abundance.

Delayed selfing: the timing of selfing relative to outcrossing is delayed, occurring after the opportunity for outcross pollen receipt has passed at the end of anthesis.

Herkogamy: the spatial separation of pollen presentation and pollen receipt within a flower in space, namely the position of the stigma relative to the anthers.

Inbreeding depression: it is reduced to fitness of inbred relative to outbred progeny, namely the deleterious effect of inbreeding on progeny performance.

Mixed mating: hermaphrodite plant species reproduce by both self- and cross-fertilization.

Monadelphous column: filaments are united to form one set or tube, high or low anthers forming part of the tube are placed below the stigmas.

Pollinia: Pollinium, or plural pollinia, is a coherent mass of pollen grains in a plant.

Reproductive assurance: self-fertilization ensures seed production when pollinators and or potential mates are scarce.

Style curvature: when the pollinators are absent or scarce, the un-pollinated style bends to anthers.