

红豆蔻专性异交主导下的主动自交机制

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摘要: 主动自交一直以来都被认为是植物在面对极端环境, 例如传粉者稀缺的情况下所采取的繁殖保障机制, 它不利的一面主要表现为近交衰退和花粉或胚珠贴现。在姜科植物部分属, 如山姜属和豆蔻属当中, 存在着一种被称作花柱卷曲 (flexistylous) 的特殊机制。过去的研究认为这种机制的意义在于避免自交和性别干扰, 但是根据我们的野外观察, 如果下垂型花朵的花粉在其花柱开始向下弯曲时仍然留在花药之上, 柱头就很有可能接触到花粉从而完成主动自交。为此我们探索了红豆蔻 (*Alpinia galanga*) 的繁殖系统, 研究结果表明: (1) 红豆蔻自交亲和, 并存在一定的近交衰退; (2) 上举型为异交, 较之下垂型投入了更多的资源; (3) 上举型的花粉胚珠比显著低于下垂型, 由于红豆蔻的胚珠数是固定的六枚, 故两种表型花粉胚珠比的差异反映了其花粉数目的差异。这可能与上举型仅仅为下垂型提供花粉, 而下垂型除为上举型提供花粉外, 还需要有足够的花粉以备自交所需。因此, 红豆蔻当中存在主动自交机制, 并且其花柱卷曲机制在避免不必要的自交和性别干扰的同时, 也为作为繁殖保障所必需的延迟主动自交创造了有利条件。同时通过控制主动自交发生的时间达到避免三种功能之间的相互冲突。这种只在姜科植物当中存在的特殊机制充分展示了其进化过程中对不利环境的适应。

关键词: 红豆蔻; 主动自交; 繁殖保障; 花柱卷曲; 适应性

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Autonomous Self-pollination under Dominant Flexistylous Outcrossing Mechanism in *Alpinia galanga* (Zingiberaceae)

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Abstract: Autonomous self-pollination is considered to be a reproductive assurance mechanism for plants when facing extreme environments that scarce in pollinators, and it also has disadvantages like inbreeding depression and pollen/ovule discounting. In several genera of Zingiberaceae like *Alpinia* and *Amomum*, a specialized mechanism named flexistylous exists, precedent studies consider its significance as preventing unnecessary self-pollination and sexual interference. However according to our field observation, if pollen grains on cataflexistylous (CATA) flowers remain on the anther when the style curves downwardly, potential exists that the stigma contacts the pollen, thus autonomous self-pollination occurs. Here, we studied the breeding system of *Alpinia galanga*, the results show that (1) *Alpinia galanga* is self-compatible in which inbreeding depression occurs to some degree; (2) The anaflexistylous (ANA) morph of *Alpinia galanga* allocates more resource into outcrossing than the CATA morph; (3) The P/O ratio of the ANA morph is significantly lower than that of the CATA morph, as *Alpinia galanga* has constant six ovules in each ovary, the significant difference in P/O value reflects the contrast in pollen production. This phenomenon may be interpreted as the ANA morph provides pollen exclusively for the CATA morph. But the CATA morph,

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despite delivers pollen to the ANA morph, also need its own pollen for potential autonomous self-pollination. In summary, autonomous self-pollination exists in *Alpinia galanga*, and while flexistylous functions to avoid unnecessary self-pollination and sexual interference, it also provides advantages for delayed autonomous self-pollination as a necessary reproductive assurance and preventing conflict among these three major features by controlling the time of autonomous self-pollination. This peculiar mechanism in *Alpinia galanga* thoroughly demonstrates its adaptation to unfavorable surrounding during the evolutionary process.

Key words: *Alpinia galanga*; Autonomous self-pollination; Reproductive assurance; Flexistylous; Adaptive significance

Self- and cross-pollination are two primary reproductive strategies in plants. In self-compatible species, self-pollination always coexists with cross-pollination (Lloyd and Schoen, 1992), comprising mixed mating system. The recurrent mixed mating mechanisms consist of pollination by flowers on the same plant including autogamy and geitonogamy, plus the combination of cleistogamy and chasmogamy, along with facilitated autogamy (Richards, 1986). Among those varying mating systems, the autonomous self-pollination is relatively less common, which occurs in flowers that are able to cross-pollination, whose occurrence does not need facilitation from anything but the flower itself. Autonomous self-pollination can be categorized in terms of the beginning time relating to outcrossing into three types: prior, competing and delayed autonomous self-pollination (Lloyd and Schoen, 1992), in which, the delayed mode, comparing with the other two, for its occurrence succeeds the culmination of outcrossing, has the least impact on cross-pollination.

With regard to the adaptive significance of autonomous self-pollination, Darwin (1876) claims the mechanism ubiquitously functions as a reproductive assurance in the situations where scarce pollinator activity, harsh environment or competitors for pollinators exist. For instance, *Epipogium roseum* (D. Don) Lindl., an obligate self-pollinated saprophytic orchid, its breeding system may possibly be evolved due to its ephemeral flowering period owing to its saprophytic nature, plus inhabiting in understory that suffers from paucity of pollinator activity since being achingly shaded (Zhou *et al.*, 2012). Though plants benefit from the reproductive assurance introduced by autonomous self-pollination, they are handicapped

by its drawbacks primarily involving inbreeding depression, namely the self-pollinated offspring is lower in fitness than those pollinated by allogamy, alongside ovule discounting, that is autogamous pollinated ovules, which produce low-fitness seeds, compete for resources with other cross-pollinated ones that produce normal offspring, decreasing fitness of the latter.

The ginger family, Zingiberaceae, is an outcrossing dominated group, exhibiting various breeding systems (Gao *et al.*, 2005a,b; Wang *et al.*, 2005; Gao, 2008) where multifold species harbor autonomous self-pollination. For instance, *Roscoea schneideriana* (Loesener) Cowley, an alpine species, achieves its self-pollination process by means of arching its terminus of style towards the anther at the incipience of blooming (Zhang and Li, 2008); Additionally, *Caulokaempferia coenobialis* (Hance) K. Larsen performs autogamy via secreting two oil-like pollen drops through the dehiscence of two pollen sacs which fuse into a larger one and gradually slide towards the horizontally grown stigma (Wang *et al.*, 2005).

In a few genera of Zingiberaceae, e. g. *Alpinia* and *Amomum*, there is a mechanism called flexistylous (Li *et al.*, 2001a,b; Gao *et al.*, 2005; Li and Xia, 2006; Liu *et al.*, 2007; Liu, 2007; Chen and Li, 2008), plants with this mechanism have anaflexistylous (ANA) and cataflexistylous (CATA) individuals within population, each individual have only one kind of flowers. The style tip of ANA morph curves downwards and being receptive in the morning with pollen sacs remain intact, and the flower is in its female state at this time. Then the style tip bends upwards until situated on the upper side of the

anther in the afternoon, following the dehiscence of pollen sacs, dispersing pollen and the flower is in its male state. The other morph, CATA, displays the very reverse pattern of ANA morph. Moreover, all individuals of identical morph in a population are synchronous in stylar movement, hence preventing pollen deposition onto stigmas of the same morph, fulfilling outcrossing. Therefore, flexistylous, the idiosyncratic strategy, realizes dichogamy by setting the flower in disparate sexual states through different periods of its lifetime, together with implementing herkogamy via relative movement of style and anther (Cui *et al.*, 1995; Li *et al.*, 2001a; Sun *et al.*, 2007, 2011).

As for the adaptive significance of flexistylous, precedent studies solely regarded it as evading self-pollination through synchronous heterodichogamy (Li *et al.*, 2001b). Nevertheless, recent studies (Sun *et al.*, 2007, 2011) proposed that merely fostering outcrossing is inadequate for understanding the adaptive significance of flexistylous. First, dichogamy is sufficient for hindering autogamy, thus it seems redundant for the flower to bear herkogamy. Furthermore, the style of ANA morph, after the stigma having received pollen in the morning, will curve to upper side of the anther in the afternoon where is inaccessible to pollinators, which is also superfluous if just for avoiding self-pollination. Nonetheless, exclusively dichogamy in the absence of herkogamy is not efficacious in preventing sexual interference, Sun *et al.* (2011) substantiated that stylar movement is capable of preventing interference between male and female functions as well as increasing pollen output, consequently increasing male fitness via comparing the seed paternity of CATA morph as pollen receptor with the stylar movement of supplier ANA morph had been restrained with the CATA seed paternity with normal provider ANA flower and found significantly less seed set in manipulated group.

Conventionally, taxa bearing flexistylous are considered to be outcrossing-dominated, even those carrying a portion of autogamous features are considered

as sporadically occurred (Chen and Li, 2008). However, according to our field observation, the CATA morph of greater galangal (*Alpinia galanga* (L.) Willd.) curves its style so extensively that the stigma is able to touch the pollen sacs. Thus if no pollinator visitation occurs during its male period, the pollen would remain on the pollen sacs, as a consequence autonomous self-pollination is in prospect. In addition, *Alpinia galanga* blossoms during the monsoon season, when pollinator activity has been limited by the weather, hence autonomous self-pollination may be chosen as reproductive assurance. Whereas many studies on flexistylous in company with some peculiar autogamous mechanisms in Zingiberaceae have been published and dramatic advance been acquired, the adaptive significance of autonomous self-pollination under dominance of flexistylous, an unparalleled strategy in ginger family, is awaiting further investigation. This study aims at answering two questions: (1) Does the CATA morph of *Alpinia galanga* possess autonomous self-pollination mechanism? (2) If so, what is the adaptive significance?

1 Materials and Methods

1.1 Study Object

The greater galangal (*Alpinia galanga* (L.) Willd. (Fig. 1: A–B), a rhizomatic perennial in ginger family (Zingiberaceae) which habitually inhabits in understory, may erect as tall as 2 m. It flowers from April to September, the raceme holding more than 200 flowers raise from the apex of pseudostem, each flower is made up by an apical petal and two lateral ones plus a pair of degenerated staminodes and a labellum with elongated stem. Seed pods are scarlet when thoroughly ripen. *Alpinia galanga* distributes in tropical secondary forests and has been semi-cultivated by ethnic minorities in Southeastern Asia like Dai for culinary use as an essential flavor, thus being economically significant.

The study employed cultivated communities grown in partly shaded understory of rubber plantation at Xishuangbanna Tropical Botanical Garden (XTBG)

(21°55'N, 101°15'E) as the research material.

1.2 Methods

1.2.1 *Basic reproductive features* One flower was randomly specified in each morph and their changes with time, especially the angle between style and

anther, were recorded and photographed sidewise at one hour interval for 26 hours from 6:00 am in the first morning, with pollinator behavior as well as blooming pattern of flowers in the vicinity also observed and documented concurrently.



Fig. 1 A. *Alpinia galanga* (L.) Willd., showing the plant and habitat; B. *Platynopoda magnifica* pollinating *Alpinia galanga*, showing massive pollen grains on its back; C-E. Status of a single ANA flower in the morning, afternoon and night of the same day; F-H. Status of a single CATA flower in the morning, afternoon and night of the same day, showing the stamens of both morphs have bent towards the labelum at night, preventing pollinators from pollinating the flowers; I. If pollen remains on the anther of CATA flower after the style curves downwards, it is possible that stigma contacts the remaining pollen, completing autonomous self-pollination; J. The infructescence of *Alpinia galanga* in its natural state; K. Infructescence of bagged ANA morph, on which no flowers set fruit; L. Infructescence of bagged CATA morph, showing some fruit set but much less than the natural state, suggesting autonomous self-pollination occurred with inbreeding depression

1.2.2 Floral characteristics One flower per inflorescence was designated at random in different plants of both morphs for more than 20 inflorescences, each part of which was measured with a vernier caliper. Additionally, on each individual of either morph one single flower bud that about to open in the next morning was arbitrarily cut off at dusk, later the ovaries and anthers were isolated and fixed by 2 mL of 70% ethanol in two Eppendorf tubes respectively for Pollen/Ovule (P/O) ratio measurements under stereoscope, more than 20 plants per morph were sampled for the experiment.

1.2.3 Bagging experiment Inflorescences with few or no blossoms opened were randomly selected and blooming flowers were removed, the numbers of remaining flowers were counted. Pollinators were precluded from visiting the flower by bagging the inflorescences with fine nylon mesh bags. More than 30 inflorescences were processed in either morph. Besides, more than 30 extra inflorescences in each morph were also marked at the same time with flowers on which counted as the control group. Infructescences were collected one month after all flowers faded and fruit set, seed set along with average seed set per fruit were counted, in which fruit set was measured via dividing overall fruit set per inflorescence by total number of flowers on the same inflorescence, while seed set was defined as division of the collective number of ovules produced by the actual number of normally-developed seeds of identical inflorescence, and seed set per fruit was equal to average number of seeds yielded by a single fruit in a single inflorescence.

1.2.4 Data analysis All the statistical tests were implemented in R by *t*-test, and the outcomes of bagging experiments were plotted in Microsoft Excel, the photos of relative movement between style and anther was processed in Adobe Photoshop for measuring the angle between the two floral organs, then the result was plotted in R.

2 Results

2.1 Basic reproductive features

The flower bud of *Alpinia galanga* cracks in ap-

proximately 20 pm at dusk, and completely opens in roughly 4 am of next morning, at about 5 am the pollen sacs of CATA morph dehisce with pollen start to spread out. The style of ANA morph commence rising at nearly 12 pm in the midday, while that of CATA morph begins to bend down at 1 pm, the ANA morph does not release pollen until 15 pm or so. Each flower lasts one day, self-compatible and nectar-providing. The result of stylar movement of the two morphs through time is shown in Fig. 2. Anthers of both morphs snap to the labellum at night, shielding from pollinator contact (Fig. 1: C-H). In CATA morph if pollen still stay on the anther when style curves downwards, possibility exists that stigma contact the pollen, hence autonomous self-pollination takes place (Fig. 1: I).

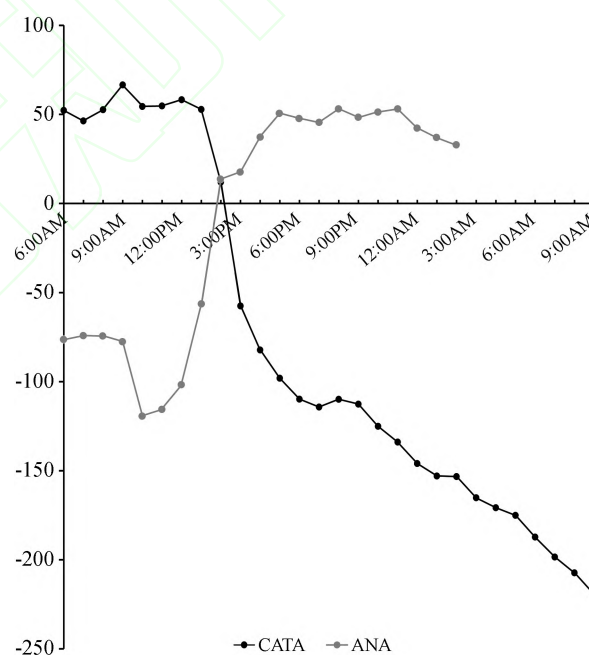


Fig. 2 Change of the angle between style and stamen over time, in which the value is positive when the style positions above the stamen, and negative when below

2.2 Floral characteristics

The result of floral characteristics are demonstrated in Table 1, showing the ANA morph significantly outcompetes its counterpart in features of flower parts like flower pedicel length, ovary size, labellum stem as well as filament size, but the CATA morph is significantly higher in P/O ratio.

Table 1 The result of floral character measurement of two morphs of *Alpinia galanga*

Characteristics	ANA		CATA		<i>t</i>	<i>df</i>	<i>P</i>
	mean	se	mean	se			
flowers per inflorescence	210	10	207	10	-0.1977	75	0.8348
pedicel length	15.464	0.449	11.114	0.312	8.1716	58	3.16E-11
ovary length	5.537	0.128	4.303	0.087	8.1959	58	2.88E-11
ovary diameter	3.170	0.055	2.644	0.028	8.9424	58	1.65E-12
corolla tube diameter	2.985	0.049	2.715	0.062	3.3462	41	0.001762
top petal width	6.86	0.167	6.375	0.078	2.7887	58	0.007146
side petal width	4.98	0.129	4.675	0.088	2.0062	58	0.04951
labellum stem length	11.277	0.326	9.761	0.234	3.8648	58	0.0002832
filament thickness	0.674	0.010	0.627	0.014	2.4946	58	0.01548
pollen/ovule ratio	2288	90	2847	114	-3.7896	43	0.0004649

2.3 Pollinator behavior

The chief pollinator of *Alpinia galanga* is carpenter bee (*Platynopoda magnifica*) (Fig. 1B). When visiting the flowers of one inflorescence, it does not switch to another inflorescence until all opened flowers on the current one have been visited, during its visitation on one inflorescence it starts from the lower flowers and moving upward. One flower can be visited several times by more than one pollinators during its lifetime and conspicuous variance exists in the duration of visitation on flowers by a pollinator.

2.4 Bagging Experiment

None of the bagged ANA group set any fruit ($0.00 \pm 0.00\%$ ($N=32$)) (mean \pm SE), consequently its seed set rate and seed set per fruit are all zero (Fig. 1K). Yet bagged CATA group produces the fruit set of ($9.78 \pm 1.71\%$ ($N=35$, Fig. 1L), the *t*-test yields $t=5.4726$, $df=65$, $P<0.0001^{***}$. On the other hand, the natural ANA renders ($44.5 \pm 2.4\%$ ($N=41$)) while the CATA delivers ($40.8 \pm 2.3\%$ ($N=36$, Fig. 1J), the outcome of *t*-test is: $t=1.1222$, $df=75$, $P=0.2654$ (ns), the ramification of comparison between CATA natural and bagged groups is $t=10.775$, $df=69$, $P<0.0001^{***}$. With regard to seed set, ANA natural group gives out ($19.7 \pm 1.0\%$ ($N=38$)) and CATA natural group ($27.8 \pm 2.2\%$ ($N=35$)), while CATA bagged produces ($6.70 \pm 1.26\%$ ($N=30$)). The *t*-test demonstrates that in between natural ANA and CATA groups $t=-3.438$, $df=71$, $P<0.01^{**}$, and

CATA natural and bagged groups $t=8.0023$, $df=63$, $P<0.0001^{***}$ (Fig. 3A); As for seed set per fruit, the natural ANA and CATA groups return 2.90 ± 0.17 ($N=38$) and 3.89 ± 0.14 ($N=35$) respectively. In contrast, the output of CATA bagged group is 3.27 ± 0.27 ($N=30$). The outgrowth of *t*-test is: natural ANA vs CATA: $t=4.3825$, $df=71$, $P<0.0001^{***}$, CATA natural vs bagged: $t=2.1149$, $df=63$, $P<0.05^*$ (Fig. 3B).

3 Discussion

Alpinia galanga harbors representative flexistylly mechanism, both ANA and CATA morphs occur in every population. The outcome of floral characteristics measurement evidently claims that ANA flowers sampled significantly surpass its counterpart in flower pedicel length, ovary size, labellum stem as well as filament size, these increased floral investments may be deemed as the ANA morph exceeds the CATA morph in expenditure in floral presentation, which can be ascribed to that ANA morph is only able to process cross-pollination so it has to attract more pollinators for reproductive success.

In accordance with classic P/O value theory (Cruden, 1977), those who bear a lesser P/O value are prone to self-pollination and vice versa. Nonetheless, as there are only fixed 6 ovules in each ovary of *Alpinia galanga*, therefore the ANA morph, with smaller P/O value, disperses less pollen than its counterpart that maintains a larger P/O value. This may

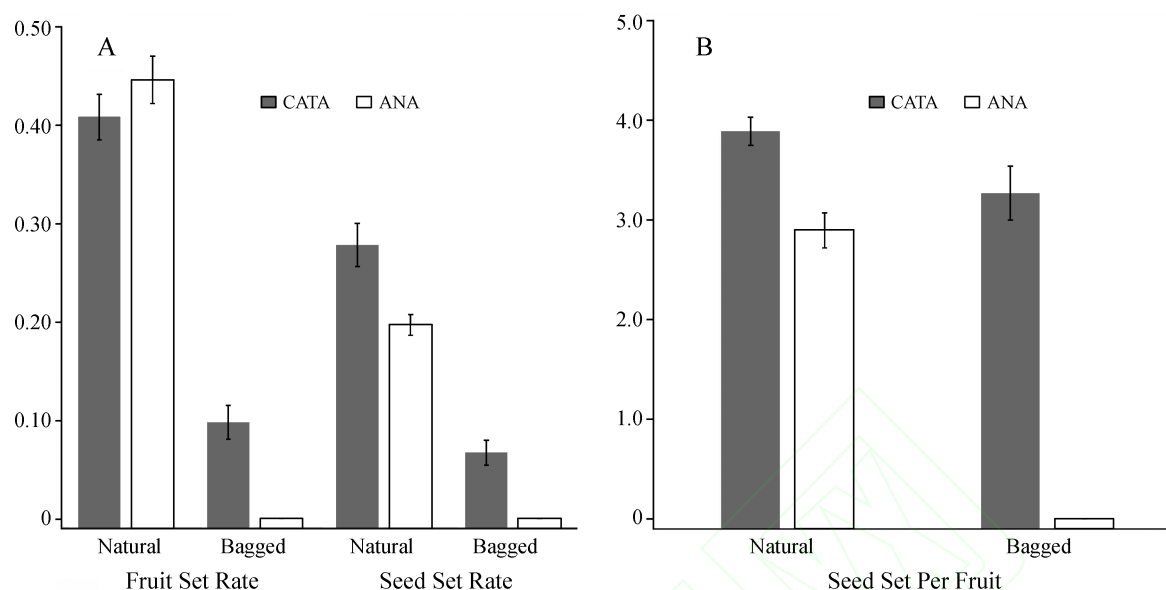


Fig. 3 A. Fruit and seed set rate, in which the bagged ANA group doesn't set any fruit so its data in fruit and seed set rate plus seed set per fruit are all zero; B. Seed set per fruit of *Alpinia galanga*

be attributed to the fact that there are no other pollen receptor of the ANA morph except the CATA morph, and despite satisfying the ANA morph for outcrossing, pollen from CATA morph are prepared for delayed autonomous self-pollination when pollinator activity is insufficient, so the CATA morph allocates more resource in generating pollen. As a result, the fact in *Alpinia galanga* that is seemingly contradict to the classical theory exactly champions our viewpoint that the ANA morph inclines to outcrossing while its counterpart favors self-pollination. Herein the P/O value, in consonance with what Queller (1984) proposes, mirrors the sexual allocation of the plant.

Moreover, through our observation, the ANA-morph disperse pollen at roughly 15 am, when pollinators are far less active than the morning, suggesting pollen limitation lies in the CATA morph. The ANA morph, however, is at its female state in the morning when pollinators are vigorous, giving rise to more pollen reception that pertinent to high fruit set. Furthermore, in association with the floral measurement data mentioned previously, the ANA flower produces notably less pollen than the CATA morph, but carries significantly larger-sized ovary, indicating in *Alpinia galanga*, the ANA morph invests more

resource in female function in guaranteeing offspring fitness, while its counterpart allocates more energy in male function (pollen production), embodying the functional diversification in the two morphs of *Alpinia galanga*, that is, the ANA morph is more outcrossing biased for providing excellent offspring while its counterpart is self-pollination inclined as reproductive assurance and serves as the only pollen supplier for the ANA morph. The ANA morph is able to spend more energy in nurturing ovaries that guarantees offspring fitness because of the CATA morph provides pollen exclusively for the ANA while satisfying its own demand for autonomous self-pollination. Accordingly the resource distribution pattern of *Alpinia galanga*, in company with its mixed mating system, are optimum measurements for adapting the surrounding and maximizing the benefit of plants. In *Alpinia galanga* populations investigated, the ANA invests more in cross-pollination and female function, its counterpart are self-pollinated as a reproductive assurance while providing more resource in male function. Considering the flower of *Alpinia galanga* lasts only one day, plus the anther snaps to the labellum, impeding pollinators from getting into the right place and pollinate the flower. *Alpinia ga-*

langa, for ensuring reproductive success, ingeniously circumvents unnecessary or detrimental autogamy and sexual interference (Sun *et al.*, 2007, 2011) while accommodating for necessary delayed autonomous self-pollination as reproductive assurance by a single mechanism, which is the result of long term evolutionary process in coping with unfavorable conditions in its surrounding.

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