Coexistence mechanism of two closely related Commelina species under reproductive interference

勝原，光希

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論 文 内 容 の 要 旨

氏　　名　勝原　光希
専　　攻　人間環境学専攻
指導教員氏名　丑丸　敦史

論文題目（外国語の場合は，その和訳を併記すること。）

Coexistence mechanism of two closely related *Commelina* species under reproductive interference
(繁殖干渉下における在来近縁ツユクサ属２品種の共存機構)

論文要旨

To reveal the mechanisms explaining why so many plant species coexist in the field has been a most important theme in the ecology, because plants are a keystone group supporting biodiversity and ecosystem functions as primary producers. In the closely related flowering plant species, sharing the same pollination niche makes coexistence difficult due to pollinator mediated competition, which is often referred to as reproductive interference. Therefore, two or more closely related plant species have been thought to can coexist only with pollination niche partitioning, often with floral trait displacement such as color, shape and/or flowering phenology, otherwise they are mutually exclusively distributed.

It has been suggested that self-pollination can mitigate the negative effects of reproductive interference by sympatrically distributed relatives. Experimental studies using planted array of close relatives showed that selfing species (species predominantly producing seeds via autonomously self-pollination) did not suffer from reproductive interference by congeners. The recent studies have suggested that prior autonomous selfing rather than delayed selfing can mitigate a reduction in seed production via reproductive interference (the

pre-emptive selfing hypothesis).

Although the pre-emptive selfing hypothesis may enhance our knowledge on the coexistence mechanism of closely related species under reproductive interference and the mating system evolution in flowering plants, there are some issues that should be examined before generalizing the relationship between reproductive interference and selfing. First, previous studies that detected negative effects of reproductive interference were mainly based on experimental arrays where the relative densities and spatial distributions were arbitrarily determined although experimental approaches often over- and underestimated the effects of reproductive interference. Second, the reported selfing species has small and inconspicuous flowers with less frequent pollinator visits compared to outcrossing congeners. Clarifying the mechanisms how selfing mitigate reproductive interference is difficult in such cases, because these selfers rarely received heterospecific pollen grains due to very low pollinator visits. Finally, adding the viewpoint of eco-evolutionary dynamics including changing densities of coexisting species through successive generations and the cost of selfing will greatly improve our understanding for the relationship among reproductive interference, species coexistence and selfing. Relative population density compared to competing species is related with the strength of reproductive interference, and thus can strongly affect selection pressure for the evolution of self-pollination. Meanwhile, the costs of selfing, such as inbreeding depression and ovule discounting, should be taken into account when considering the evolution of autonomous self-pollination. Threfore, to examine the possibility of evolutionary rescue by self-pollination and resultant coexistence of competing species under reproductive interference, these factors and their interactions are desired to be

together considered.

The aim of this thesis is to test my hypothesis, which is developed based on the pre-emptive selfing hypothesis: the evolution of prior autonomous self-pollination mitigates the negative effects of reproductive interference and consequently promotes coexistence of closely related species sharing the same pollination niche. For this purpose, I conducted empirical ecological and genetic studies on sympatrically distributed two native *Commelina* species, *Commelina communis* (Cc) and *C. c.* f. *ciliata* (Ccfc) and a theoretical study based on eco-evolutionary dynamics framework.

In chapter 2, I described the mutual reproductive interference between sympatrivally distributed two native *Commelina* species in detail under natural condition. Cc and Ccfc exhibit largely niche overlap, such as flowering phenology, habitat preference and pollinator composition. Based on field survey, I found that Cc population exhibit dominant distribution compared to Ccfc population in meso-scale spatial distribution and the negative effect of reproductive interference on seed production is stronger in Ccfc than in Cc although heterospecific pollen transfer via pollinator is evenly occur. By combining with field survey and glasshouse experiment, I also showed that prior self-pollination can assure seed production even with heterospecific pollen deposition and asymmetry of reproductive interference might be explained by the higher ability of prior self-pollination in Cc. These findings suggested that the prior autonomous selfing could mitigate the negative effect of reproductive interference from competitor and likely promote coexistence of these species.

In chapter 3, I investigated how reproductive interference affect selfing rate and genetic structure in Ccfc based on population genetics method using microsatellite

markers. I firstly developed microsatellite marker for Ccfc using next generation sequencer. My results showed sympatric population (Ccfc distributes with Cc) tend to exhibit higher population inbreeding coefficient and selfing rate (close to 1.0) than allopatric population (only Ccfc distributes) although an increase of relative flower abundance of Cc does not affect seed's selfing rate of Ccfc flower in sympatric population. It might be interpreted that outcrossing individual of Ccfc in the sympatric population is already excluded out via selection due to reproductive interference. Besides, genetic diversity and genetic differentiation from other populations were not difference between sympatric and allopatric populations. It might be interpreted that study Ccfc individuals in sympatric populations produced seeds predominantly though selfing.

In chapter 4, to test my hypothesis in the context of eco-evolutionary dynamics, I developed individual based model in which two plant species that share the same pollination niche and can evolve prior autonomous selfing compete against each other in the form of mutual reproductive interference. Based on model simulations with various parameter condition, my model revealed that the evolution of prior selfing can promote the coexistence in the presence of mutual reproductive interference when pollinator availability and strength of inbreeding depression were intermediate levels. Meanwhile, the coexistence rarely occurred without the evolution of prior selfing. With the variable inbreeding depression (inbreeding depression decreases with increasing the population's selfing rate), coexistence was facilitated by the evolution of prior selfing in wider conditions than with the fixed inbreeding depression. Especially when the strength of inbreeding depression gently decreased and pollinator availability was

intermediate level, the coexistence with evolutionary rescue often occurred and stably continued for very long-term. Totally, my simulation results showed the long-term coexistence with evolutionary rescue occurred with moderate pollinator limitation, variable and moderate levels of inbreeding depression, the relatively higher initial prior selfig rate and the presence of reproductive interference.

In the chapter 5, I discussed the contribution of my thesis for understanding of species coexistence mechanism and evolution of plant mating system as a general discussion. In the context of species coexistence, coexistence of closely related species is traditionary thought to be difficult due to sharing very similar ecological niche, such as habitat, resource demand and reproductive biology. although previous theoretical studies have shown stable coexistence under reproductive interference must require niche partitioning between and among competing species, my model results showed evolutionary rescue by prior selfing could occur and stably continued and cause long-term coexistence under reproductive interference even when two species is completely same in ecological features (chapter 4). Some studies have shown that spatial structure can enable the coexistence under reproductive interference because reproductive interference promotes spatial segregation among competing species and make interspecific competition relatively weaker than intraspecific competition. In my study plant system, Cc and Ccfc exhibited largely niche overlap, such as flowering phenology, habitat preference and pollinator composition (chapter 2). My results suggested Cc and Ccfc might exhibit conspecific aggregation in micro (individual) spatial scale distribution but not in meso (population) scale, so contribution of conspecific aggregation for coexistence of Cc and Ccfc is still unclear (chapter 2).

Because I found strong mitigation effect of prior selfing against reproductive interference and relatively higher population's selfing rates in sympatric Ccfc populations, I believe the evolution of prior autonomous selfing play a more important role for the coexistence of Cc and Ccfc. It is thought to have large applicability for other systems because evolution of shift to higher selfing rate is often occur in many independent plant taxa. This coexistence supported by the evolution of prior selfing without any kinds of niche partitioning and specific spatial structure should improve our understanding especially on coexistence between closely related plant species.

In the context of evolution of plant mating system, I revealed the coexistence under reproductive interference can occur only with co-evolution of extremely high prior selfing rate in both competing species ($\geq$ 0.9; chapter 4). This result is consistent my empirical study in which selfing rate is estimated close to 1.0 in Ccfc population growing with Cc although selfing rate of Cc have been not estimated (chapter 3). That it should be noted, however, Cc and Ccfc have been visited frequently by pollinators such as bumble bee and syrphid fly (chapter 2). Flowers of Cc and Ccfc flowers with showy blue petals and yellow stamens were visited frequently by pollinators such as bumble bee and syrphid fly even in sympatric populations (Chapter 2). The features are inconsistent with typical predominately selfing flowers that evolve under pollinator limitation, strong inbreeding depression and transfer advantage of selfing gene. Recent studies, including my studies, pointed out that reproductive interference via interspecific pollen transfer can facilitate the evolution of selfing. My results suggest that the high selfing rate in showy flowers might be often found in the presence of competing species sharing the same pollinators.

Finally, I discuss a few suggestions of future studies. First, based on field survey of various populations, to examine the relation among inbreeding depression, selfing rate and population size must be important to test my model prediction, which is strength and dynamics of inbreeding depression are important determinants for coexistence possibility of competing species (chapter 4). Second, to clarify the floral traits related with prior selfing rate is thought to be also important although there are some candidates such as degree of herkogamy and dichogamy. Especially, the evolvability of these traits, such as heritability and genetic variation in the field, is quite important for deciding whether the evolutionary rescue can increase population size of minority species before competitive exclusion.

（注）3,000～6,000字（1,000～2,000語）でまとめること。

［課程博士用］

論文審査の結果の要旨

氏　　名	勝原光希		
論文題目	Coexistence mechanism of two closely related *Commelina* species under reproductive interference （繁殖干渉下における在来近縁ツユクサ属2品種の共存機構）		
判　　定	合　　格		
審査委員	区　分	職　名	氏　　名
	主　査	教授	丑丸敦史
	副　査	教授	近江戸伸子
	副　査	准教授	高見泰興
	副　査	准教授	源利文
	副　査	農学研究科 准教授	杉浦真治

要　　旨

本博士論文では、近縁植物2種が繁殖干渉下で共存するメカニズムに関する新仮説「各種における先行自家受粉による自殖（先行自殖）の進化が、相互の繁殖干渉の影響を緩和することで、長期的な近縁植物種の共存を促進しうる」を提唱し、その検証を行っている。生態学では、Georgy Gauseによって競争排除則が提唱されて以降、同じ生態的地位（ニッチ）を持つ多種の共存は困難であるとされてきた。そのため、非常に良く似たニッチを共有する近縁種は共存しづらいと考えられてきた。しかし、野外では近縁種の共存がしばしば観察されており理論からの予測と一致しないことが指摘されてきた。本論文では、送粉ニッチ（開花場所・時期や送粉者）を共有する近縁植物品種（ツユクサとケツユクサ）の野外での共存を説明する新仮説の提唱及びこの仮説の妥当性や一般性の検証を行うことを目的としている。本論文は5章構成で、序論である第1章、野外でみられるツユクサとケツユクサの共存が先行自殖により可能になっていることを明らかにする第2-3章、数理モデルシミュレーションにより新仮説の妥当性を検証した第4章、以上の個別研究で得た結果の総括を行う第5章、謝辞、引用文献及び付録資料から構成されている。

まず、第1章では、近縁植物種の共存を説明する仮説やその検証に関する既往研究について総括し、送粉ニッチが重なる近縁種では、形質置換による送粉者の使い分けや生育環境や開花時期のずれを進化させることで送粉過程におけるニッチ分割が実現した場合にのみ共存が可能になること、このようなニッチ分割なしでは強い繁殖干渉によっていずれかの種の絶滅が起こることが説明されている。また、近年になって、「先行自殖を行う種は他種からの繁殖干渉の影響をあまり受けず、他殖を行う近縁種と共存可能である」ことが明らかになってきたことを紹介している。ただし、既往研究で対象とされた自殖植物は花が小さく、送粉者に訪花されない種であるため、繁殖干渉を軽減する要因が、他種花粉の柱頭付着機会の減少なのか、先行自殖による繁殖保証であるのか、は解明されてないことを指摘している。これらの既往研究の総括を受け、本章では「繁殖干渉下にある近縁種は、各種における先行自殖による繁殖保証の進化により、ニッチ分割を伴わずとも長期的に共存する」という仮説を提唱し、以下の章における研究の意義を説明している。第2章では、同所的な共存がしばしば観察される在来近縁品種であるケツユクサとツユクサを調査対象として研究を行い、両品種ともに野外環境下で同じ送粉者に頻繁に訪花され、他品種からの繁殖干渉により種子生産が低下すること、先行自殖によって繁殖干渉の効果が軽減されうることを明らかにしている。第3章では、ケツユクサを対象として、自身が開発したSSRマーカーを用いてツユクサと共存する集団と単独で分布する集団の自殖率の推定・比較を行っている。その結果、共存集団の自殖率は単独集団のものより高い傾向があることを見出し、ケツユクサはツユクサによる繁殖干渉下では、専ら自殖により集団を維持していることを示している。第4章では、個体ベースモデルを用いて、全く同じ送粉ニッチを持ち、双方向の繁殖干渉がみられる2種が、先行自殖の進化により長期間共存しうるかについて、また2種の共存が可能となる条件について、理論的な検討を行っている。本モデルのシミュレーション解析の結果、繁殖干渉下の2種の長期的な共存には先行自殖の進化が必須であること、2種共存は送粉者制限があり、自殖率と近交弱勢の強さに負の相関があるという条件下で実現しやすいことを明らかにしている。第5章では、以上の個別研究の結果を受けて、1章で提案した仮説の妥当性や一般性、今後の発展について議論し、研究全体を総括している。

以上の通り、本博士論文は申請者が提案する新仮説について、野外調査や栽培実験、分子遺伝学的実験、数理モデル解析等の複数のアプローチから検証を行い、その妥当性を示している。既存の理論では説明困難とされてきた近縁植物種の共存を説明する新たなメカニズムを提唱した点、また仮説検証を包括的に行ったという点において世界的に見ても非常に高い新規性が認められる。

なお、本論文を構成する各章（第2-4章）は個別の投稿論文としてまとめており、第2章はFunctional Ecology誌に、第3章のSSRマーカーの開発に関する研究はGenes & Genetic Systems誌にて公表されている。第3章の残り部分および第4章は投稿論文を書き上げ投稿直前である。下記に発表論文の詳細を示す。

Katsuhara, K. R. and Ushimaru, A. (2019) Prior selfing can mitigate the negative effects of mutual reproductive interference between coexisting congeners. Functional Ecology 33: 1504-1513.（査読付）

Katsuhara, K.R., Nakahama, N., Komura, T., Kato, M., Miyazaki, Y., Isagi, Y., Ito, M. and Ushimaru, A. (2019) Development of microsatellite markers for the annual andromonoecious herb *Commelina communis* f. *ciliata* (Commelinaceae). Genes & Genetic Systems 94:133-138.（査読付）

以上より、学位申請者の勝原光希は、博士（理学）の学位を得る資格があると認める。

レフェリー付きの論文の発表について，記載すること。