

国立科学博物館国際シンポジウム 2009  
National Museum of Nature and Science International Symposium 2009

# 生物の相互関係が創る多様性

Origin of Biodiversity by Biological Interactions

要旨集 Abstracts



What is biodiversity?

主催：独立行政法人 国立科学博物館  
平成21年11月21日 土 → 23日 月  
国立科学博物館 [新宿分館]

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# 生物の相互関係が創る多様性

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# 虫媒花は色と形をいかに使うか

田中 肇

日本花粉学会評議員

虫媒花とは、昆虫に花粉を運ばせて受粉する花の総称であり、昆虫に効率よく花粉を運ばせるための様々な構造や機能が備わっている。そのため、植物の種ごとに異なった姿を見せているが、一方では花粉を運ぶ昆虫の形態や習性に適合するため、系統的にかけ離れた植物でありながら、よく似た色彩や構造をもつようにもなっている。ここでは、そのような虫媒花の多様な姿と類似性の一端をみることにする。

## 花の色彩

花を見るとき、もっとも気づきやすいのは色彩である。これは昆虫に花を発見してもらうためのディスプレイとして、さらに花の種類や状態を認識してもらうためのサインとして機能していると考えられる。しかし、昆虫に色を区別する能力がなければ、花の色には意味がないことになるが、その能力の存在はフォン・フリッシュの実験に始まる多くの研究により確認されている。

昆虫が色を区別している様子は、野外で蜜を吸う行動を観察することで、容易にみてとれる。ランタナの花はいくつもの小さな花の集りであるが、その小さな花の中で昆虫が口吻をさしこむのは、黄色を帯びた花である。それらは花粉を出し雌しべが受粉可能な生殖活動中の花であり、蕾や生殖活動を終えた花と異なり、蜜を分泌している。また、オランダガラシの花は中心部が緑色をおびた花が生殖活動をしており、生殖活動を終えた花は中心部が褐色になる。咲いている花の数では褐色をおびた花のほうが多いのだが、訪れたハナアブ類が口吻を差し込んだ花の8割は、生殖活動中の緑色をおびた花であったと報告されている。



図1. ランタナとイチモンジセセリ



図2. オランダガラシとシマハナアブ

また昆虫は紫外線も色として知覚するため、ヒトには一色に見える花も、中心部に紫外線を吸収した模様がある花が多く知られている。この模様は昆虫に蜜の存在位置を知らせる機能があると考えられている。それらは紫外線写真を通して見ることができる。



図3. アブラナの紫外線と可視光の写真

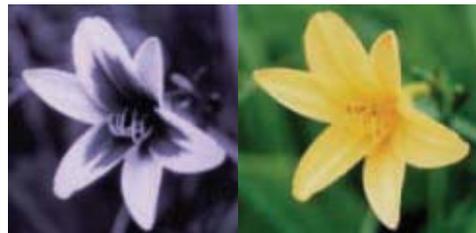


図4. ニッコウキスゲの紫外線と可視光の写真

## 花の構造と機能

花の構造は、昆虫により確実に受粉するように仕組みられている。そのいくつかの例を挙げる。

春の野を彩るオオイヌノフグリの花は、細くしなやかな花柄に支えられているため、昆虫が花に止まると昆虫の重みで花柄が曲がる。すると昆虫は落ちないように花の中心に突き出ている雄しべ雌しべに脚をかけるが、雄しべの根元は細く曲がりやすいため、必然的に先端の葯を昆虫自身に引き寄せることになり、花粉が運ばれる。しかも、体重わずか0.03gのホソヒラタアブによっても、この受粉の仕組みは機能することがわかっている。

コオニユリやヤマユリの雄しべはT字形をしており、花粉の入った葯は重心近くが長い花糸に支えられ、ふらふらと動きやすくなっている。花粉を媒介するのは、花びらの基部近くから分泌される蜜を吸いに来る、アゲハチョウの仲間である。チョウは花びらや雄しべを足場に蜜を吸うが、そのとき葯に触れる。しかし、チョウの羽は鱗粉におおわれており、水やゴミがつきにくい。チョウにとってはゴミである花粉もつきにくいいため、葯は動いて掃除機の吸込み口のように、チョウの羽にピタッとよりそう形になり、花粉をつける。しかも花粉は他の花より粘りけがあり、よく付着する。

同じアゲハチョウに花粉を媒介してもらおうツツジの仲間は、ラップ形の花びらの背側に細長い管があり、その管の底に蜜をたくわえている。雄しべ雌しべは長くアゲハチョウが蜜を吸おうとすると、それらの先が触れる。ツツジの花粉は粘着糸と呼ぶ細い繊維で花粉をつづり、その繊維でチョウの脚などからみつき、一度に多量の花粉が運ばれるようになっている。

ツツジ類以外にも粘結糸をもつ花粉をつくる植物が知られており、その一つがマツヨイグサの仲間である。マツヨイグサ類の花にも細長い筒がある。夜咲く花にはチョウと同じ仲間のが訪れる。やはり羽などは鱗粉におおわれているため、花粉がつきにくい。そのため花粉は粘結糸でつづられ、一度に多量に運ばれるよう仕組みられている。ツツジとマツヨイグサとは類縁関係がかけ離れた植物だが、鱗翅目に属するチョウやガを送粉者とするとき、同じような手法を用いていることがわかる。



図5. ツツジの花粉



図6. オオマツヨイグサの花粉

春に咲くカラスノエンドウとムラサキケマンの雄しべ雌しべは、花弁につつまれている。昆虫の餌となる花粉や蜜は、雄しべ雌しべを入れている花弁を押し下げないと採取できない。そのような作業ができるのは、ハナバチ類のみであり、花弁を押し下げることでハチを介して花粉が授受され。そして花は、共に紫色の花弁とよく似た色である。

虫媒花の多様な色彩、形態、機能などは、花と昆虫が相互に選択しあう中から生み出されたのであり、ここではそのほんの一端に触れたにすぎないが、花と昆虫との共生関係は興味のない奥深い世界である。

# 誰がために花は咲く

## ～動物をあやつる信号機としての花～

川窪 伸光

岐阜大学・応用生物科学部

私は、近年、植物の繁殖器官である「花」を、従来とは異なった視点で解析する試みをしてきました。それは「花」を、花粉媒介者(送粉昆虫)の行動をコントロールする「信号装置」として捉え、花粉媒介者誘引機能の実態を明らかにする試行錯誤といえるでしょう。この講演では、その試行錯誤に基づき、浮かび上がってきた花の送粉昆虫誘引機能を紹介します。実は、一連の研究の成否は「花」に飛来する送粉昆虫の「花着地前」の飛行行動を3次的に徹底的に解析できるかどうかにあります。この研究の方法論が確立できれば、従来の植物の繁殖生態学、特に送粉生態学に新たな地平を提供するとまで私は信じて試行錯誤してきました。私と研究室の学生達との悪戦苦闘の研究過程を、花と虫の映像を交えて紹介いたします。

### ●研究の背景1: 「花」の特性

生物界をひろく見渡すと、植物の「花」の機能には、他の生物にはない、非常に特異な点があります。それは、雌雄配偶子の出会いを、非生物的现象や特定動物種の行動を利用して実現している点です。自らが移動できない

固着性の植物は、異個体との花粉のやりとり(花粉媒介)を、空間を移動する風や水、そして多様な動物を利用し実現しています。この花粉媒介の現象は、動物全般では見られないものです(表1)。

つまり、固着性である植物の「花」は、本来の繁殖機能を果たすために、交配相手へのディスプレイとして働くのではなく、雄性配偶子のカプセルである花粉の授受を担う動物種へのディスプレイとして機能しているのです。したがって「花」は動物の行動をコントロールする信号装置と考えるのも良いのではないのでしょうか。

### ●研究の背景2: 訪花後観察ではなく訪花前観察が必要

頭花植物は、目立つ場所(花)に、動物種の餌となる花蜜や花粉(報酬)を準備し、それら報酬を与える代償として、花粉の授受を実現するように、「花」を進化させてきたと考えられています。したがって従来の研究観察は、もっぱら、「どのような花が、どのような動物を引き付けているか?(動植物種間対応関係)」や、「訪れた動物は、花の上でどのような行動をとるのか?(授受粉に有効な動物種の特定)」そして「その行動は、花の提供する報酬の量と質にどのように関係するのか?(最適な資源配分の推定)」などの疑問に基づいて行われてきました。

しかしながら、このような研究は、動物が「花」を訪れた後(着地後)の観察(訪花後観察)のみに基づいてきました。それは、「花」の重要な特性である「いかんにして動物種の行動をコントロールしているのか?」についての直接的(具体的)観察データ(訪花前観察)の採集が非常に難しいからです。なぜなら、花粉媒介動物種の多くが、飛来する昆虫や鳥であり、その注目すべき行動は、目にもとまらぬ瞬時の現象で、それこそ熟練者にとっても肉眼での詳細な観察は不可能だからです。

### ●悪戦苦闘方法: ビデオカメラで送粉昆虫の飛行軌跡を解析

そこで、訪花昆虫たちの花に着地する寸前の飛行行動の解析のために、2台のデジタルビデオカメラを用いて、動画として連続画像を採集して、そこから、昆虫たちの飛行軌跡を3次元座標として把握することを始めまし

表1. 動植物間における有性繁殖器官の機能対比

有性繁殖過程	動物種 哺乳類の一部 交尾器(交尾器)	植物種 頭花植物 花(生殖器)
1	配偶子(卵・精子)の生産	配偶子(胚珠・花粉)の生産
2	配偶者選択(異性の誘引)	花粉の移動 動物(送粉者)・風・水等の利用
3	交尾	受粉
4	受精	受精
5	幼体発生	幼体発生

た。観察対象として用いた植物はアレチヌスビトハギで、訪花昆虫はバラハキリバチモドキです。

●悪戦苦闘結果：送粉昆虫の訪花には作法がある

講演でお見せするようなビデオ動画を、1秒間におよそ30枚の静止画とるカメラとして扱くと、動画から図1のような訪花昆虫の行動が把握できます。一枚一枚の静止画から昆虫の頭と尻の座標を読み取り、2台のカメラ

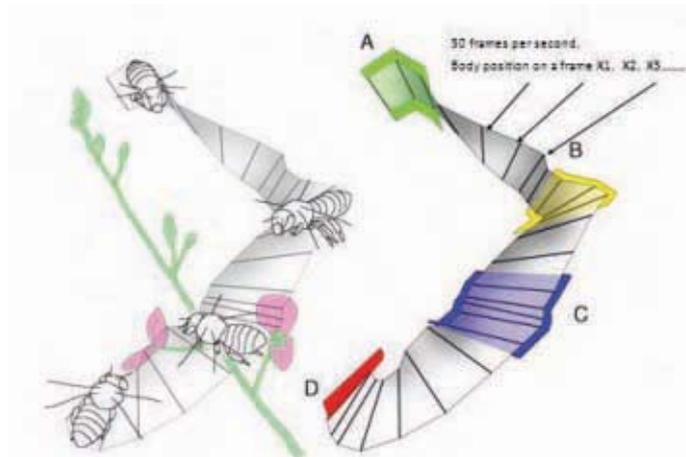


図1. ビデオカメラ録画による解析

が同時(同期的)に記録した2枚の静止画を対応させ、コンピュータの仮想3次元空間内に昆虫の位置を再現し、飛行軌跡を展開していくのです。このような3次元データから、昆虫個体の飛行速度・加速度、飛行高度、飛行姿勢、飛行ルートなどが明らかになり、そして昆虫が見ているであろう方向が推定できます。今回の一連の研究から明らかになってきた現象は、私たちヒトがレストランを探す行動に対比して考えると判りやすいでしょう。

私たち人間は、食事するレストランを、まず大きな看板(広告塔)や香りによって、大まかなレストランの位置

を把握します。次に、その看板や香りの元へ移動・正面入り口へ接近し、店の雰囲気や、提供される料理の質を、レストランの外部から観察推定するでしょう。そして、入店するか否かを決断するのです。一方、レストランは、これらの広告塔や店のデザイン、そして料理の内容の表示などにおいて工夫を凝らし、私たちを誘引しています。ですから、このような一連の誘引過程において、私たちはレストランに行動をコントロールされて、入店させられていることとなります。

解析の結果は、この関係をまったく彷彿とさせるものでした。花に飛来する昆虫の行動からは、1.「花」の発見、2.「花」の位置認識、3.「花」の方向認識、4.「花」の価値評価の段階を想定できそうでした。一方、それらに対応するように、植物側は、A.「花」の存在、B.「花」の位置、C.「花」の開花方向、D.報酬の在処(訪花価値)を情報として提示しているように思えました(表2)。これらの過程で、昆虫は空中姿勢などに見られる段階的な行動を示し、その過程には【花接近決断点】とでも言える行動の分岐点や、そして【花着地最終決断点】と言えそうな行動分岐点が存在するようにも思われました。

表2. 虫媒送粉系の仮想的な花信号系列

訪花昆虫の行動	虫媒植物の花信号
前花からの離脱	
1. 次の花の探索(発見)	「存在」
2. 花の位置認識	「位置」
3. 開花方向確認	「開花方向と着地地点」
4. 花の訪花価値評価	「報酬」
訪花もくろみ離脱	

花信号の各要素は同時に発信されている(チャンネルがある)。が、受粉者の受信能力(距離感・判断力)で各要素の受信能力が異なるのかもしれない。

●今後の展開：花の役割の全容が知りたい

「誰がために花は咲く」のか？そもそも花は私たちヒトを喜ばせるために咲くものではありません。植物個体、自らの有性繁殖を成功させる装置として花は咲くのです。つまり花を咲かせる多くの植物たちは、花で昆虫たちを引き寄せ、花粉や花蜜を与えつつ、花粉を他株の花へ運ばせ、また花粉を他株から受け取り、繁殖を成功させているのです。その過程で「美しい花」は、昆虫たちの行動を操作する単なる信号機なのです。現在の送粉生態学では、多くの研究者が多方面から研究を進めており、花と昆虫の関係が、驚くほど巧妙で、多様性に富み、地球上の生物間相互作用の要であることを明らかにしています。私も、これまでの試行錯誤をさらに展開させることで、花と昆虫たちの未知の関係が見えてくるかも知れないと思うと、これからの研究にワクワクします。

# 菌類が動物・植物とつくる共生ネットワーク

細矢 剛

国立科学博物館

菌類（きのこ・カビ・酵母）は自然界では生物遺体の分解者として知られる。このような生き方は死物を腐らせているので「腐生」と呼ばれる。しかし、菌類は腐生にとどまらず、生きたさまざまな生物と相互作用をもち、この関係は「寄生」や「共生」として知られている。共生関係とは、関係する両者が互いに利益を得るような関係をさすが、時として、片方だけが利益を得る（が、相手には不利益は生じない）場合も含まれる。本演題では、とりわけ多様な菌類と植物の関係を中心に、菌類がさまざまな生物と展開する多様な関係を紹介する。

植物は独立栄養なので、その生活には菌類を必要とすることはないように思われる。しかし、陸上植物の大部分は、根において菌類と「菌根」という構造を形成し、菌類の助けにより栄養を摂取する。菌根とは、植物の根を菌糸がとりまいて変形したり（外生菌根）、その細胞の内部に菌糸が入り込んで特有の構造が形成されるもので、菌類は無機塩類を植物に供給し、植物は光合成産物を与えることによって共生関係が成り立っている。植物の一部には、菌類が存在しないと生育がおぼつかないものもある。菌根は様々な分類群の菌群にみられる。また、多くの植物において形成されることが知られており、普遍的な共生系であることが判明している。なかには、葉緑体を失うことにより光合成が行えず、菌類に絶対的な従属栄養性をもつ、いわば、菌類を食べる植物さえ存在する。

単細胞の藻類は菌類との共生によって、地衣体を形成する。菌類は、藻類の光合成産物を享受し、藻類は菌類と共存することによって生育場所を確保する。両者が共生することによって、極地や山火事の後など、劣悪な環境の中で生き延びることができると考えられている。

一見健全に見える植物の地上部（茎・幹・葉など）にもエンドファイト（内生菌）と呼ばれる菌類が内在し、その中には、抗真菌物質を生成するなどして、宿主を他の菌の攻撃から守るような関係も存在する。エンドファイトの一部は、宿主に病原性を示す場合もあるため、これは寄生・共生の中間的な形ということができる。しかし、どのようなきっかけで共生的な状態から寄生的な状態に移行するのか、病原的なポテンシャルをもちながら宿主を健全な状態に保つことができるのか、など基本的な問題は解明されていない。エンドファイトの一部は根にも存在しており、これが植物の栄養摂取に寄与することも最近判明してきた。

菌類が関係を持つ相手は、植物だけではない。熱帯域にはシロアリの仲間がきのこの菌糸を栽培し、シロアリの食料とし、きのこはそのシロアリの排泄物を栄養として育つというような共生関係が存在する。

菌類の一部は、時に動植物に寄生し、病害などの被害をもたらす。寄生とは、自分にとっては利益になるが、相手にとっては有害となる関係である。多くの菌類は植物の病原体として知られており、特に栽培植物に対する寄生は人間にとっても重要な問題である。また、菌類は動物（特に昆虫）の病原菌として知られている。冬虫夏草はその代表であり、全世界で約 800 種が知られている。

共生や寄生は、相手を選ばないわけではない。多くの場合、菌類は特定（あるいは特定の

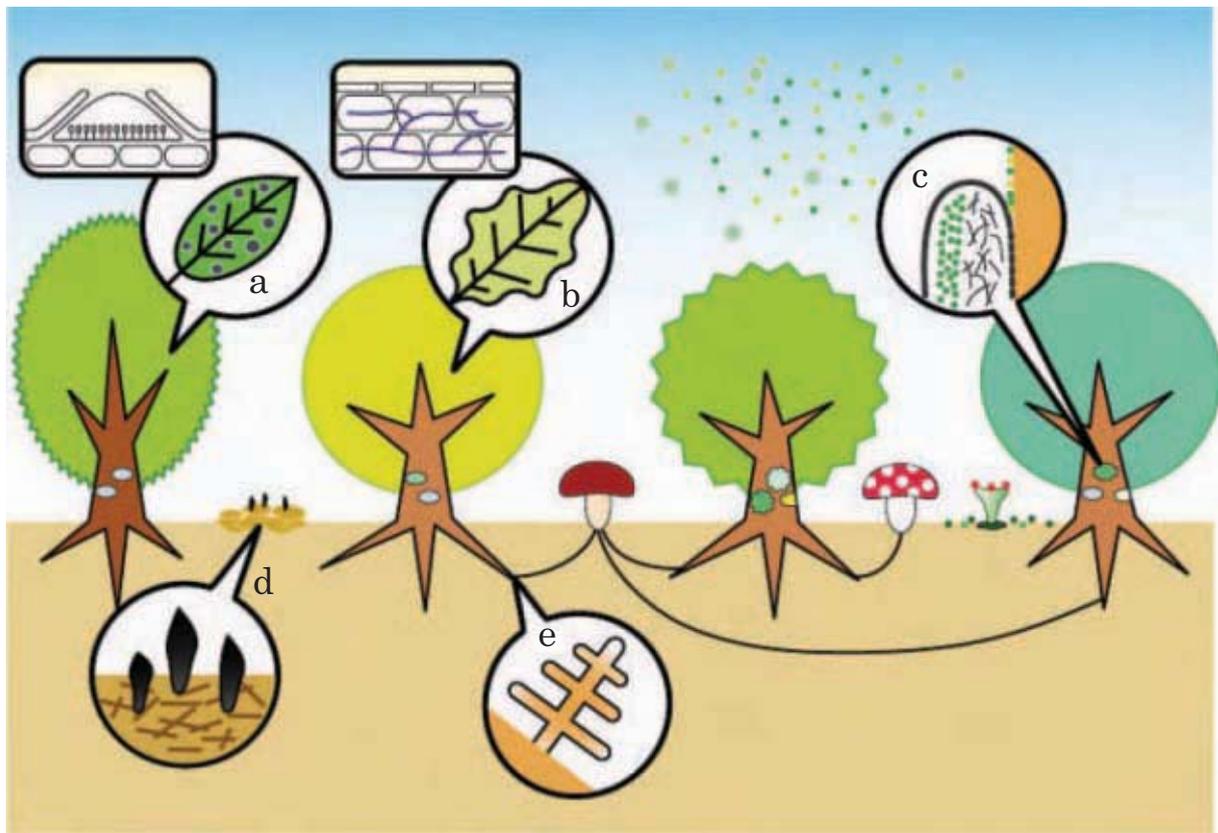
範囲)の相手と寄生・共生関係を結ぶ(選択性)ことがしばしばあるので、両者はある種のパートナーシップを築いていると考えられる。

一方、植物遺体の分解を行う菌類の中にも、植物基質に対して選択性をもつものが知られており、菌類と植物のパートナーシップは、植物の生死に関わらず存在するといえる。

さらに、菌類と植物の関係は一対一ではない。一つの菌類が複数の動物・植物と関係を持ち、一つの動植物が複数の菌類と関係を持って網状に広がっている。このような関係は広い意味での共生ネットワークと理解される。このように、菌類を介してさまざまな生物が網状につながって共生ネットワークを形成している。

菌類の寄生・共生というはたらきは、人間にとっても無縁ではない。動物病原性のカビは、生物農薬として開発されているし、植物の生育を助けるような菌類についても、生育促進剤としての研究が進んでいる。菌類を介した共生関係の研究は基礎研究の観点からも、応用研究の観点からも、今後ますます重要なものとなる。

#### 菌類と植物が繰り広げる共生ネットワーク



この図には菌類と植物が広げるさまざまな共生関係を図示した (a.病原菌による植物の病斑;b.一見健全な植物体に潜むエンドファイト;c.菌類と単細胞藻類が形成する地衣体。樹木の幹上にしばしば見られる;d.腐生による植物遺体の分解;e.外生菌根を形成している菌類。その多くはきのこである。)

# 太陽を目指して泳ぐ共生藻をもつクラゲたち

並河 洋

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水中には、コンブやワカメなどの大型の藻類以外に、顕微鏡でなければ見ることの出来ない微小な藻類が生息しています。微小な藻類は、光が届くところならば地球上いたるところに分布しており、その中には動物体内で生活している仲間もいます。このような藻類は、棲みこんでいる動物との間で互いに栄養を与え合う関係（共生関係）をつくっており、共生藻と呼ばれています。

## いろいろな海産動物と共生する褐虫藻

水中に棲む動物と共生関係にある微小な藻類には様々な種類があります。その中には、サンゴと共生関係にあることでも知られる褐虫藻がいます。褐虫藻は、渦鞭毛藻類の仲間で、約 10 マイクロメートルの大きさです。名前の通り褐色をしている藻類で、動物の体内では、球形となっています。褐虫藻は、共生している動物の代謝産物である二酸化炭素や窒素化合物（アンモニアなど）を使って、動物の体内で盛んに光合成を行い増殖します。一方、光合成でできたエネルギーの余剰分は、共生している動物が使い、その動物の成長を促します。

## サンゴと褐虫藻の共生関係

サンゴにはたくさんの種類があり、種によって深海から浅い海まで様々な海底に生息しています。褐虫藻は、太陽がさんさんと降り注ぐ暖かな浅い海にすんでいるサンゴ類に共生しています。褐虫藻と共生しているサンゴ類は、造礁サンゴ類と呼ばれています。「造礁」とは石灰岩でできたサンゴ礁をつくることです。つまり、造礁サンゴ類は、褐虫藻が光合成で生み出したエネルギーを使って石灰質の骨格を成長させ、サンゴ礁をつくり上げていくのです。造礁サンゴ類が褐虫藻なしでは生きられないことは、最近マスコミにも取り上げられるようになった「サンゴの白化」という現象が如実に示しています。サンゴの白化とは、造礁サンゴ類に水温の上昇などのストレスがかかると、褐虫藻がサンゴ体内から抜け出してしまい、白色のサンゴ骨格が透けて見えることを言います。造礁サンゴ類は、白化した状態が長く続くと生命を維持できず、死滅してしまうと考えられています。

## クラゲと褐虫藻の共生関係

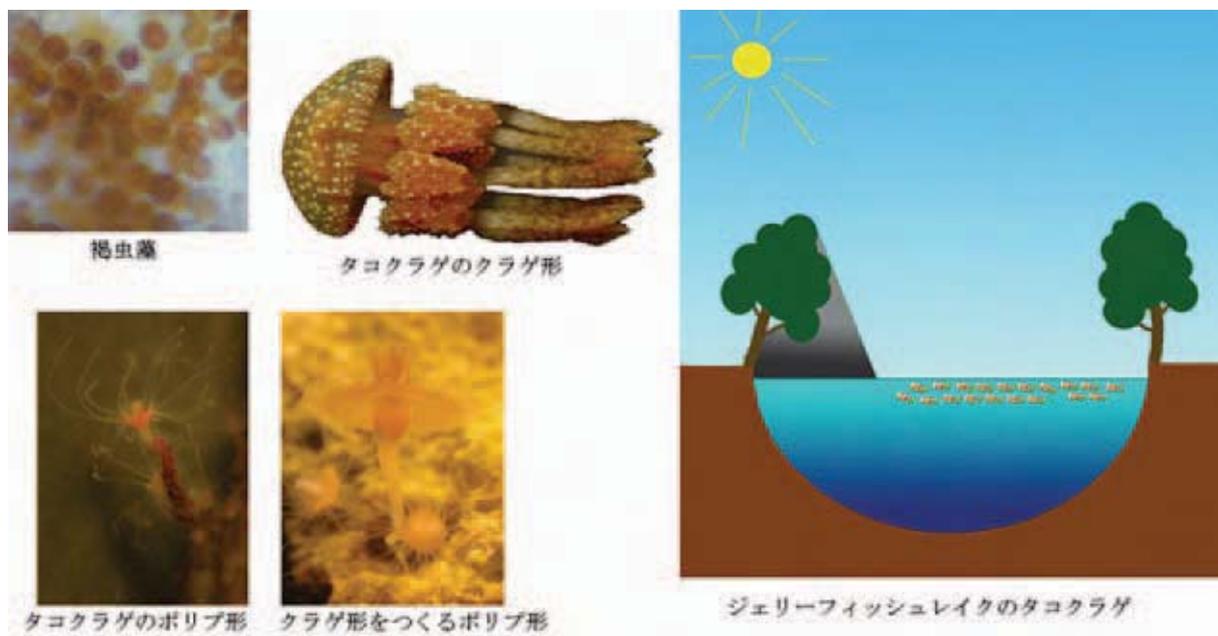
サンゴの仲間であるクラゲ類にも、褐虫藻と共生するものがあります。クラゲ類にとっては、成長するためだけでなく、子孫を残していくためにも褐虫藻の存在が重要であるという報告があります。

クラゲ類には、生活史の中で、イソギンチャク類やサンゴ類のように岩などに固着して生活する『ポリプ形』と水中を漂って生活する『クラゲ形』とがみられます。我々が普段見ているのはクラゲ形です。ポリプ形は大きさ 1・2mm 程度の小さなものです。クラゲ形には雌雄の区別があり、受精した卵は、プラヌラと呼ばれる幼生になります。プラヌラは、岩などに付着すると変態し、ポリプ形になります。ポリプ形は無性的にポリプ形をつくとともに、季節が来ると無性的にクラゲ形をつくります。一見姿形の異なるクラゲ類とサンゴ類とが同じ仲間ということ不思議

議に思われる方も多いと思われませんが、ポリプ形をみることで、同じ仲間であることがわかるのではないのでしょうか。クラゲ類においても、サンゴ類やイソギンチャク類のように基本的にポリプ形で生活しています。サンゴ類やイソギンチャク類においては、生殖巣がポリプ形の体内にできるのですが、クラゲ類は生殖巣を体外につくることを選んだ動物といえます。つまり、クラゲ形は、「泳ぐ生殖巣」であり、季節が来ると咲く植物の花のようです。

褐虫藻が体内に共生するクラゲ類は、造礁サンゴ類と同じように、暖かな海に棲んでいます。種類としては、タコクラゲやサカサクラゲなどが知られています。これらのクラゲ類において、受精卵やプラナラには褐虫藻が存在していません。褐虫藻は、プラナラの変態後、ポリプ形の口から体内に入ります。しかし、ポリプ形の維持には褐虫藻の存在はあまり関係ないようです。ポリプ形は、エサを捕まえる毒針（刺胞）をもち、小さな動物プランクトンを捕まえて食べることができるからです。しかし、褐虫藻が共生していないと、ポリプ形はクラゲ形をつくることができないようです。つまり、褐虫藻は、クラゲ類が子孫を残す上で非常に重要な役割を演じていることになります。ポリプ形からつくられたクラゲ形は、ポリプ形と同様に刺胞を使って捕まえたエサを食べて成長しますが、褐虫藻のつくるエネルギーも利用しているために光に集まる習性があります。

パラオ諸島のジェリーフィッシュレイク（クラゲ湖）という閉ざされた海水湖には、大量のタコクラゲが生息しているようです。これまでの研究によると、このタコクラゲは、刺胞をもたず、エサを捕まえることがないようです。つまり、すっかり褐虫藻が作り出すエネルギーに頼って生きているのです。そして、これらのクラゲたちは、ジェリーフィッシュレイクの水面で効率よく太陽光を浴びて生活できるように、太陽を目指して泳ぐクラゲたちなのです。



# Community-level plant-pollinator interactions in Asia and Oceania

Makoto Kato  
Kyoto University

Almost all parts of the land on the earth are characterized by distinctive flora, vegetation and plant–pollinator interactions. Community-level plant–pollinator interactions reflect biogeographical history of the land and history of coevolution between plants and pollinating animals, and each of them is being very unique. I review general pattern of the community-level plant–pollinator interactions in Asia and Oceania, especially focusing on tropical ecosystems.

## Tropical rain forests

Tropical rain forests in Southeast Asia are confined to peninsular Malaysia, Borneo, and Sumatra, where it is hot and humid throughout the year. The forests are dominated by the ectomycorrhizal plant family Dipterocarpaceae and are characterized by supra-annual general flowering whose rhythm is determined by the El Niño Southern Oscillation. The supra-annual general flowering causes unpredictable changes in floral resources and poses a challenge for plant-pollinator mutualisms. Because the majority of canopy trees participate in general flowering after a several-year-long non-flowering period, trees need a vast number of pollinators to outbreed. In turn, the pollinators must subsist on limited floral resources or other food sources during the non-flowering period. The potential pollinators of these canopy trees are reportedly the giant honeybee *Apis dorsata* and chrysolimid beetles.

In contrast to these canopy trees, typical understory perennials bloom on the dark forest floor throughout the year. Many have deep tubular flowers that are pollinated mainly by shade-loving, long-tongued bees of the apid genus *Amegilla*. In a Bornean rain forest, two species of *Amegilla* trapline and pollinate diverse rare flowers of Zingiberaceae, Maranthaceae, and Gesneriaceae. These *Amegilla* bees are ecological counterparts of euglossine bees in the Neotropics.

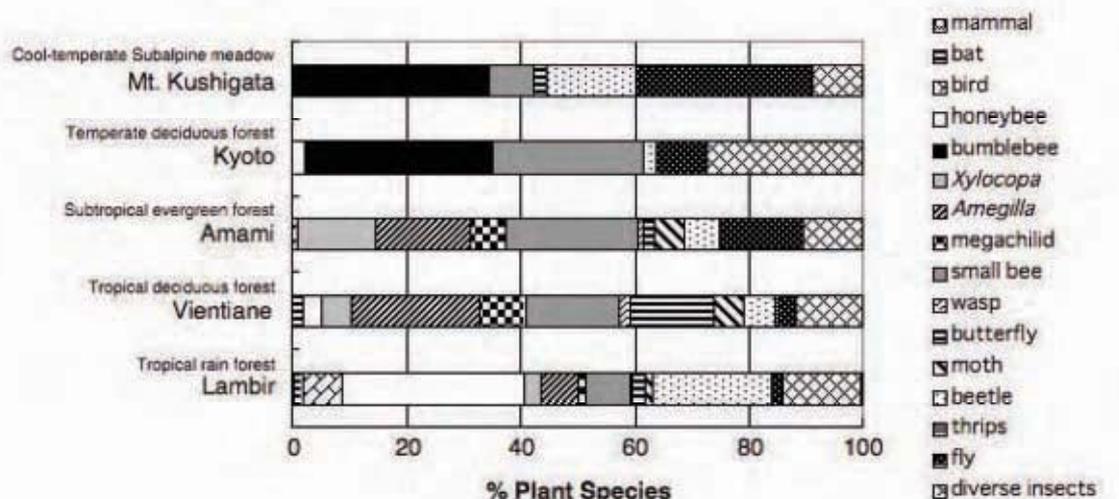


Fig. 1. Comparison of plant pollination systems among various vegetation types.

### Tropical monsoon forests

Tropical monsoon forests are characterized by an alternation of dry and wet seasons caused by changes in the prevailing wind direction. Flowering occur throughout the year, although the number of flowering plant species peak at the end of dry season. The dominant canopy trees, including Dipterocarpaceae, bloom annually at the end of the dry season. The most dominant pollination system is melittophily; *Xylocopa*, megachilids, and honeybees mainly contribute to the pollination of canopy trees, whereas long-tongued *Amegilla* bees pollinate diverse perennials with long corolla tubes.

### Forests on oceanic islands

The flora of New Caledonia is characterized by remarkably high species diversity, high endemism, and an unusual abundance of archaic plant taxa. The most dominant pollination systems were melittophily, phalaenophily and ornithophily. The prevalence of ornithophily by honeyeaters shows an ecological link to pollination mutualism in Australia. The relative dominance of phalaenophily is unique to New Caledonia, and is proposed to be related to the low diversity of the original bee fauna and the absence of long-tongued bees. Although some archaic plants maintain archaic plant-pollinator interactions, the most dominant organism observed on flowers was the introduced honey bee, *Apis mellifera*, which suggests that the unique systems of pollination mutualism in New Caledonia are now endangered by the establishment of highly invasive honey bees.

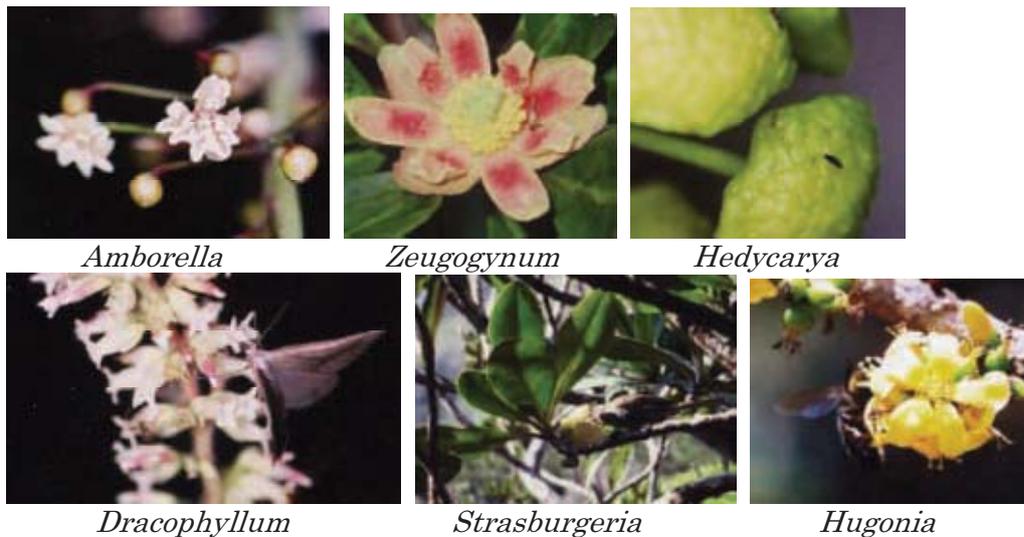


Fig. 2. Characteristic pollination systems in New Caledonia.

Bonin Islands are another example of oceanic islands harboring many endemic flora and fauna. The original bee fauna is characterized by dominance of small solitary bees as observed in Hawaii. The dominance of these solitary bees is supposed to have caused evolution of dioecy on these oceanic islands, because hairlessness of these small bees results in limited movement of pollen and consequently imposes the plants on higher cost of geitonogamy on the plants. The European honeybee was introduced to Bonin Islands in 1930s, and became naturalized on several islands. On these islands, most endemic bees became extinct, and native plant-pollinator interactions are critically endangered.

# Pollinator driven divergence among populations of South African plant species

Bruce Anderson

South Africa boasts one of the most diverse arrays of floral variation on the planet and with an astonishing variety of floral colours, shapes, scents and rewards at all taxonomic levels. Much of the variation in floral form has been attributed to pollinators whose idiosyncratic preferences and peculiarities of body shape have been the tools used to sculpt the intricate features of these flowers. When the body plans or the preferences of these pollinators are geographically variable it can theoretically result in geographically divergent selective pressures which can drive the allopatric diversification of plant traits.

I will present examples of three systems where the geographic variability of pollinator morphology or preference has resulted in variable plant traits such as corolla length, scent and colour, at the population level.



Figure 1. The long proboscis fly *Prosoeca ganglbaueri* visiting its main host plant *Zaluzianskya microsiphon*

First I present *Zaluzianskya microsiphon* (Scrophulariaceae) which is pollinated predominantly by a single species of long proboscis fly across its entire range (Fig 1). In some populations the fly has a very short proboscis ~ 20mm which it uses to probe the deep corolla tubes of *Z. microsiphon* for nectar. But in other populations the proboscides of the flies reach lengths of up to 50mm. We suggest that there is a selective pressure on fly tongues to match the tube lengths of their most important host plant in order to access their nectar most efficiently, and we also show that there is a selective pressure on plants to match their pollinators tongue lengths because it enhances the efficiency of pollen transfer. Our results suggest that there is close matching between tube and tongue length traits at each population and that this is largely a result of coevolutionary processes. The geographically variable outcomes in this system may be due to differences in both biotic (e.g. community context) and abiotic (wind and temperature) environment which could oppose coevolutionary selective pressures.

At many of the same sites, the long proboscis fly also visits a rewardless orchid (*Disa nivea*) and it attracts the flies by mimicking the colour of the nectar plant *Z. microsiphon*. Here the spur lengths of *D. nivea* also match the tongue lengths of the fly pollinator but this matching could not have resulted from coevolution because it is non adaptive for the fly to match the spur length of the orchids as the spurs hold no nectar. Instead, it is an example of trait matching by unilateral evolution where only the flower adapts to the fly but not vice versa.



Figure 2. Long tubed flowers from the north are predominantly visited by long tongued hawkmoths but the short tubed flowers from the south are predominantly visited by short tongued hawkmoths.

The second example of pollinator driven divergence is for the plant *Gladiolus longicollis* (Iridaceae) which has long corolla tubes in the north but short ones in the south. We find that these plants are pollinated by hawkmoths but that there are two distinct guilds of hawkmoth pollinators: long and short tongued moths. We find that the long tongued moths are most common in the northern parts of the country but that the short tongued moths are most common in the south, giving rise to a north-south divergence of corolla tube length (Fig. 2). We also found a zone of secondary contact where short and long tubed plants co-occur. In this zone, long tubed plants seem to be predominantly visited by long tongued moths and short tubed plants by short tongued moths. Since floral colour in these tube length morphs is identical, we were unsure of how moths could distinguish between the flowers until we examined floral scent. Here we found that each morph had a distinctive scent, which may allow for learning to develop. Different scents probably evolved in allopatry either as an adaptive response to different moth scent preferences or as neutral variation which could then have gene flow consequences upon secondary contact.



Figure 3. Red flowers and pink flowers of *Drosera cistiflora* are visited by different species of monkey beetle

The last example is of floral colour variation in the insectivorous plant *Drosera cistiflora*. The turn-over of colour in this species occurs over very small spatial scales, sometimes of just a few kilometers. Populations of *D. cistiflora* may be white, pink, purple, red, or yellow in flower colour with white being the most common and widespread. These flowers are pollinated primarily by monkey beetles which are pollen feeders, but different flower colours often seem to be visited by different species of monkey beetle. In a pilot experiment I made a reciprocal translocation experiment between flowers from a red versus a pink population. In the red population, red cuttings received most of the visits from monkey beetles, whereas in the pink population both morphs received similar numbers of beetle visitors. At each site, the composition of the beetle species differed between flower colours suggesting that colour preferences of beetles can cause assortative mating. This could result in geneflow barriers and thus contribute to the speciation process.

Since these examples are all of divergence among populations of a species, it suggests that pollinator driven divergence often occurs in concert with (and not after) the speciation process, highlighting the important role of pollinators in plant speciation.

# Why do species interact? A test of four hypotheses using *Ceropegia* (Apocynaceae) as a case study.

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## Summary

Interactions between species form the basis for community structure and ecosystem function in all terrestrial and marine habitats, via energy flow and nutrient movement between trophic levels, and top-down and bottom-up control of species' abundances. In addition, species interactions provide the impetus for the evolution of a huge range of biological novelty. In the absence of relationships such as mutualism, predation, parasitism, competition and commensalism, biodiversity would be much simpler than it is today, and the biosphere would be a very different place.

Despite this central importance of species interactions in relation to ecology, evolution and conservation biology, there is much that we do not understand about why certain species interact, why other species are excluded from those interactions, and how this in turn promotes organismal diversification through adaptation and co-evolution. In this study we test four non-exclusive hypotheses which relate to these questions, using the genus *Ceropegia* L. (Apocynaceae: Asclepiadoideae, Ceropegieae) as a case study. *Ceropegia* is a large Old World taxon of over 180 accepted species, with new species being regularly discovered. All species studied to date are pollinated by small Diptera (0.5–4.0 mm in length) from a diverse range of families that are temporarily trapped in the flask-shaped flowers. Using flower visitor data for over 60 species of *Ceropegia* across its distribution, plus a cpDNA-nrDNA molecular phylogeny of the genus, we addressed the following hypotheses:

**Hypothesis 1** – *the coevolutionary hypothesis*: the pattern of species interactions that we recognise today is the result of reciprocal evolution between two unrelated clades of organisms, such that speciation in one clade results in speciation in the second clade.

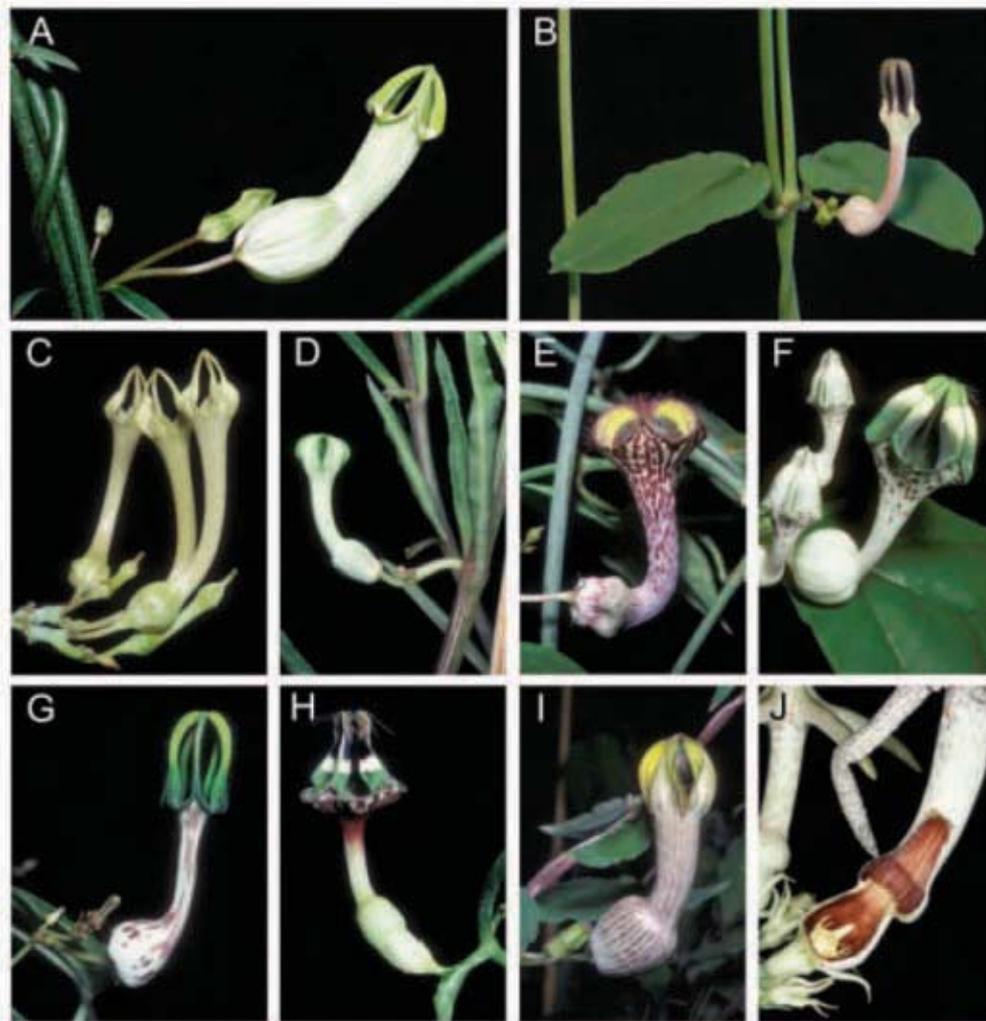
**Hypothesis 2** – *the phylogenetic hypothesis*: species are the products of the evolution of their ancestors. Thus, as well as evolving adaptations that relate to their life histories and behaviour at the current time, they are influenced by a range of phylogenetically conservative traits which (presumably) evolved in the distant ancestral past of that clade.

**Hypothesis 3 – the biogeographic contingency hypothesis:** species interact with one another on the basis of their current global and local distributions, i.e. they interact because, spatially, they can interact.

**Hypothesis 4 – the local adaptation hypothesis:** those features of the organismal phenotype which are required adaptations for a particular interaction have generally evolved locally (i.e. within the native range of the organism) in response to natural selection imposed by the interacting partner.

Our analyses find no support for Hypotheses 1 and 3 and only limited support for 2 and 4. We discuss these findings in relation to the data that will be required in the future to fully address the latter two hypotheses. Studies of species rich clades of biotically pollinated flowering plants such as *Ceropegia* have enormous potential for addressing questions relating to the biodiversity of species interactions. However the logistical difficulties of adequate sampling of pollinator observations is a formidable one, given the spatio-temporal variation in interaction structure inherent in all plant-pollinator assemblages.

**Below: Representative taxa of *Ceropegia* illustrating floral diversity in the genus** (A, *C. ampliata*; B, *C. imbricata*; C, *C. aristolochioides* subsp. *aristolochioides*; D, *C. crassifolia* var. *copleyae*; E, *C. affinis*; F, *C. sankuruensis*; G, *C. meyeri-johannis*; H, *C. denticulata*; I, *C. cufodontii*; J, *C. variegata* (basal inflation opened, exposing corona). Photographs: U. Meve.



# Does coevolution drive diversification? Evidence from the pollination mutualism between Phyllanthaceae plants and *Epicephala* moths

Atsushi Kawakita

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Coevolution is expected to drive diversification of the associated lineages because local coadaptation leads to rapid phenotypic divergence between populations. Obligate pollination mutualisms, such as the fig–fig wasp and yucca–yucca moth mutualisms, provide strong candidates for such coevolution-driven diversification scenario because (i) pollinators are highly specific to their locally adapted hosts and (ii) specialist pollinators are expected to promote reproductive isolation between locally diverged hosts. However, tests of coevolution-driven diversification have been limited because it is often difficult to distinguish the contribution of coevolution from those of other drivers of diversification (e.g., habitat specialization).

I use a phylogenetic approach to test whether specialized pollination mutualisms drive diversification of the partners in the newly discovered mutualism between Phyllanthaceae plants and *Epicephala* moths (1). The Phyllanthaceae–*Epicephala* mutualism is ecologically analogous to the fig and yucca systems in that the females of specialist seed parasites pollinate the flowers in which they lay eggs, while the emerging offspring feed on the resulting seeds. *Epicephala* pollinators are locally specific to a single Phyllanthaceae host (2,3), although a strict one-to-one rule is not perfectly met. Importantly, this mutualism evolved independently six times within Phyllanthaceae (4), allowing for multiple independent comparisons of whether sister clades with and without *Epicephala* pollination differ in their diversification rates.

I first show that host specialization in the moths is a direct result of coevolution. Host specificity of pollinating seed parasites (fig wasps, yucca moths or *Epicephala* moths) is often attributed to their inherently parasitic lifestyle because these insects are derived from herbivorous ancestors that themselves tend to be host specific. However, *Epicephala* moths exhibit distinctly higher host specificity than do their non-pollinating, herbivorous ancestors, indicating that strict pollinator specialization is the result of selection acting after the establishment of the mutualism. Chemical coadaptation is likely responsible for this specialization because closely related, sympatric Phyllanthaceae hosts have distinct floral odors, and *Epicephala* pollinators have clear preferences to the floral odor of their natal host species (5).



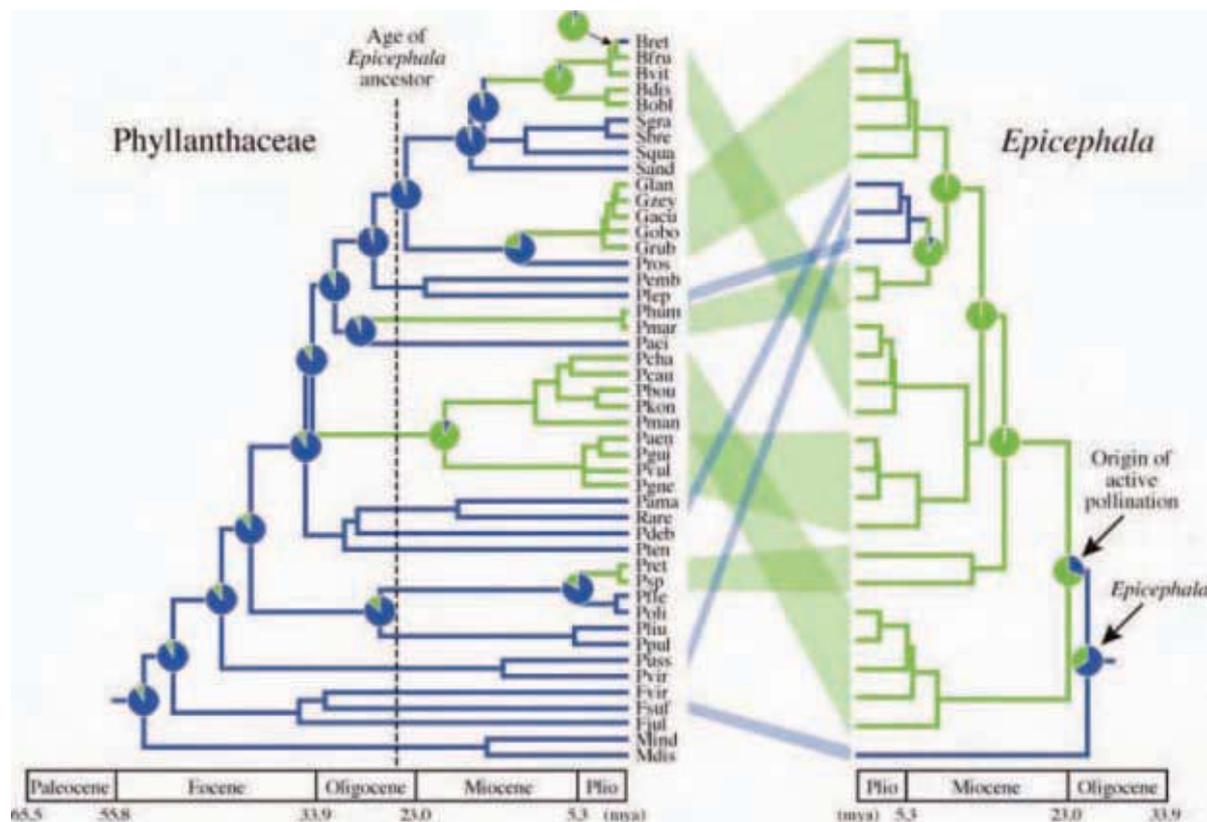
(Photo) Female *Epicephala* moth actively collects pollen with its proboscis (left, middle left) and pollinates the female flower in which she lays an egg (middle right). Hatching larva consumes two of the six seeds in the fruit (right).

Phyllanthaceae includes estimated 500 species that are each pollinated by species-specific *Epicephala* moth. These 500 species are grouped into six distinct clades on the Phyllanthaceae phylogeny. Comparisons of diversification rates between moth-pollinated and non-moth-pollinated sister clades indicate that, in all comparisons, clades with *Epicephala* mutualists have higher diversification rates than those without. Furthermore, four of the six clades with *Epicephala* pollination have significantly higher diversification rates than expected under constant diversification.

Although studies are needed to analyze precise mechanisms by which coevolution may drive diversification, available data are consistent with the prediction that specialized pollination mutualisms promote diversification of the plant and pollinator partners. Analyses of geographic variation in floral odor chemistry may be a promising avenue for future research.

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(Figure) Phylogeny of Phyllanthaceae plants (left) and *Epicephala* moths (right). Lineages involved in obligate pollination mutualism are indicated in green. Mutualism arose multiple times in Phyllanthaceae whereas active pollination behavior evolved only once in *Epicephala*.

# Speciation at a single chemical: an extensive multi-species comparison revealed a key floral volatile for pollinator isolation in Asian *Mitella*.

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**Despite its most recent origin among major land plant lineages, nearly 90% of all extant land plant species are flowering plants (angiosperms). This remarkable species richness of angiosperms is considered partly because of their predominant adaptation to animal pollination (1). In animal-pollinated plants, changes in any floral traits that influence pollinators' behavior are responsible for forming reproductive isolation and could ultimately cause speciation. Although this view may explain the accelerated speciation rate in angiosperms, little is understood about the tempo and mode of an establishment of pollinator isolation through floral trait evolution. Historically it has been considered that an accumulation of many changes of small effect, often coupled with other isolating barriers, is required for an establishment of reproductive isolation, but a few known examples of visual cues suggested otherwise, i.e., a smaller number of changes of large effect in floral coloration might be sufficient (2, 3). Here we present that, in olfactory cues, the presence or absence of even a single compound could result in a dramatic change of pollinator species and thus could dominate strong reproductive barriers of angiosperms.**

A monophyletic lineage of perennial plant *Asimitellaria* (Asian *Mitella*) shows remarkable diversity in Japan, consisting of 13 species, of which all except one species are Japan endemic. *Asimitellaria* is unique in that almost all species are pollinated only by specific species of fungus gnats with a rewarding system (4). In many populations, two *Asimitellaria* species occur in sympatry. Interestingly, they never or seldom hybridize to each other in nature while they easily produce hybrids if hand cross-pollinated (5). Field observation revealed that this clear reproductive isolation of sympatric *Asimitellaria* species is attributable to pollinator isolation, with one species being pollinated by short-tongued fungus gnats such as genus *Boletina* and *Coelosia*, whereas the other species being pollinated by a characteristic long-tongued fungus gnat species *Gnoriste mikado* (Fig. 1). As the previous phylogenetic analysis indicated that the pollinator shift between short-tongued and long-tongued fungus gnats had occurred multiple times (4), we surveyed floral traits that might have evolved in association with these pollinator shifts.

We suspected floral scent trait as the determinant of pollination specificity in *Asimitellaria*, because the flowers of *Asimitellaria* are usually inconspicuous, and the pollinator fungus gnats are most active at dawn and dusk, so visual cues seem less important. We analyzed total floral scent chemicals of >100 plants spanning 11 *Asimitellaria* species by

head-space sampling coupled with a GC-MS, and the data were averaged for each of the plant species. Using high-resolution phylogeny of *Asimitellaria* based on >8kb from nine nuclear genes, we found that the interspecific variation of total floral scent traits is indeed associated with the variation of pollinator assemblages (partial matrix correspondence test [PMCT],  $p < 0.01$ ) but not with interspecific, phylogenetic genetic distance (PMCT,  $p > 0.3$ ), suggesting that the floral scent traits have evolved rapidly as an adaptation to pollination by specific fungus gnats in *Asimitellaria*.

Accordingly, the floral scent traits were further dissected by looking at the association between the relative amount of each compound and the pollinator assemblage for each *Asimitellaria* species using phylogenetically independent contrast (6). As the result, we could determine several compounds that are significantly associated with specific pollinator fungus gnat species. Subsequently, we conducted a series of bioassay using these potentially bioactive compounds and pollinator fungus gnat species, and finally confirmed that the presence or absence of a single chemical compound determines the attraction specificity of pollinator fungus gnat species in *Asimitellaria*.

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**Fig. 1. Flowers of eight representative *Asimitellaria* species and their pollinator fungus gnats. Upper from left: the flowers of *Mitella* sp., *M. yoshinagae*, *M. acerina*, and *M. pauciflora* each pollinated by short-tongued fungus gnat species. Lower from left: *M. japonica*, *M. kiusiana*, *M. makinoi*, and *M. furusei* each pollinated by a long-tongued fungus gnat species *Gnoriste mikado*.**



# Geographic mosaic of damselfish-alga cultivation mutualisms in the Indo-West Pacific

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Kinki University

To understand biodiversity of coral reefs, one must understand the complex relationships among species. In marine ecosystems, it has been suggested that there are many cryptic species and therefore most of the species-specific interactions have yet to be discovered (Knowlton 1986, 1993, 2000, Knowlton et al. 1992). We detected cryptic species-specificity between filamentous red algae in the genus *Polysiphonia* and damselfishes (Hata & Kato 2006). Territorial damselfishes defend territories individually from invading herbivores and maintain algal farms on which they exclusively feed in the territories (Branch et al. 1992, Ceccarelli et al. 2001). Molecular genetics enabled us to detect four cryptic species of *Polysiphonia* algae, each of which were collected from territories of four different damselfish species. Notably, *Polysiphonia* sp. 1 inhabited only algal farms of one damselfish species, *Stegastes nigricans*. *Polysiphonia* sp. 1 was highly digestible for *S. nigricans* (Hata & Kato 2002). The fish weeded competitively superior algae to protect the *Polysiphonia* sp. 1, as well as chasing away intruding herbivores (Hata & Kato 2002, 2003). In this way, *Polysiphonia* sp. was exclusively protected and grown by *S. nigricans*, and *S. nigricans* fed on the alga as a staple food. Similarly, other damselfish species had other species-specific relationships with *Polysiphonia* species, none of which were abundant outside the territories. Thus, their relationship was an obligate cultivation mutualism, as seen between humans and their crops (Hata & Kato 2006).

A more recent study in the Indo-West Pacific (Mauritius, Kenya, Egypt, Maldives, Thailand, Malaysia, Okinawa and Australia) revealed that the general pattern of species-specificity between *Stegastes* damselfishes and *Polysiphonia* algae was relatively stable throughout the Indo-West Pacific, but the dependency on the specific partner varied remarkably. *Polysiphonia* sp. 1, which is dominant in algal farms of *S. nigricans* in Okinawa, Japan, was also found in territories of the same fish species in Mauritius and Australia. However, *Polysiphonia* sp. 1 did not always dominate the farms in those areas, and therefore the fish did not always depend on the alga as a staple food. In addition, three new sibling *Polysiphonia* species were found. The farms of *S. nigricans* in Egypt, Kenya and Maldives were inhabited by any one of those three indigenous *Polysiphonia* species, instead of *Polysiphonia* sp.1. Therefore, the cultivation mutualisms between the damselfish and *Polysiphonia* algae are indeed maintained through the Indo-West Pacific, with intrageneric partner shifts of crop algae in the West-Central Indian Ocean. These results imply that the cultivation mutualisms

between damselfish and *Polysiphonia* algae have coevolved under geographic selection mosaics (Thompson 2005) with host shifts across regions.

On coral reefs, several territorial damselfish species typically coexist (Sale 1979, Robertson & Lassig 1980, Waldner & Robertson 1980). Their territories occupy 11-70% of substratum and the damselfishes and the algae play key roles shaping benthic communities as well as fish communities (Wellington 1982, Sammarco & Williams 1982, Hixon & Brostoff 1983, 1996, Zeller 1988, Glynn & Colgan 1988, Ferreira et al. 1998, Hata & Nishihira 2002). Furthermore, the territories contribute ~50% of total reef algal production (Russ 1987, Klumpp et al. 1987). Understanding the diversity and patterns of interactions between damselfishes and algae will help our deeper understanding of mutualisms, which are hallmarks of coral reef ecosystems, and also bring new insights into the critical role which territorial damselfishes play in coral reef biodiversity.



Fig. 1. Territorial damselfishes and their farms. (A) *Stegastes nigricans* in Okinawa and its monocultural farm of *Polysiphonia* sp. 1; (B) *S. nigricans* in Kenya; (C) *Plectroglyphidodon leucozonus* in Egypt; (D) *S. limbatus* in Mauritius. Each individual maintain its own algal farm that is a dense stand of filamentous algae. Outside the farms algae are intensively grazed by roving herbivores and we can see bare white coral rocks.

# Long drills and thick walls in a weevil-camellia arms race

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Ever since Darwin (1862) had predicted the existence of a long-tongued moth as a pollinator of a Malagasy star orchid with 30-cm-long flowers, coevolutionary races have been posited as major processes driving the evolution of



Fig. 1. A flower of the Japanese camellia (left), copulating camellia weevils (middle), and a cross section of a camellia fruit (right) with holes made by female weevils (arrows).

exaggerated organismal morphologies (Nilsson 1988; Anderson & Johnson 2008). An ideal system for understanding the processes of such races is found in the interaction involving the camellia weevil (*Curculio camelliae*; Curculionidae) and its obligate host plant, the Japanese camellia (*Camellia japonica*; Theaceae). Females of the weevil bore into the pericarps of Japanese camellia fruit with their extremely-long mouthparts (rostra) to make holes used for oviposition. A laboratory experiment revealed that the longer the weevil rostra, female rostra were more likely to reach seeds, whereas the thicker the camellia pericarps, camellias were more likely to protect their seeds. Hence, the two traits are expected to evolve through arms races (Toju & Sota 2006).

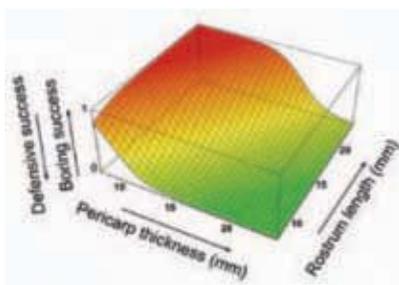


Fig. 2. Dependence of offensive/defensive success on camellia pericarp thickness and weevil rostrum length.

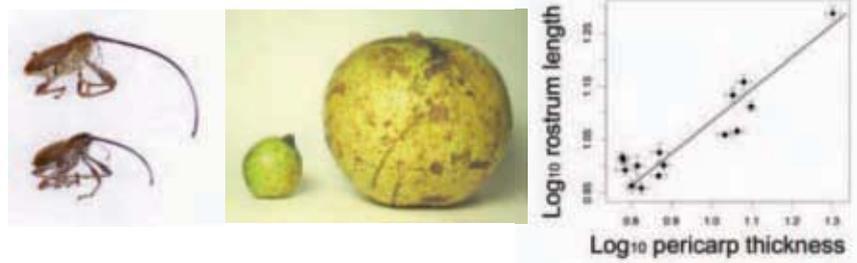


Fig. 3. Geographic variation in female weevil rostra (left) and camellia fruit size (middle). Traits of the two species are correlated with each other across populations (right). After Toju & Sota (2006).

Morphological and ecological analyses of the weevil-camellia interaction over the Japanese archipelago indicated that the coevolutionary processes were differentiated among geographic populations. There was a clear correlation between weevil rostrum length and camellia pericarp thickness across populations and both traits were exaggerated at lower latitudes. In

addition, natural selection exerting on camellia pericarp thickness through seed predation by weevils varied among populations, suggesting that inter-population variation in the camellia trait reflected geographically varying coevolutionary interactions.

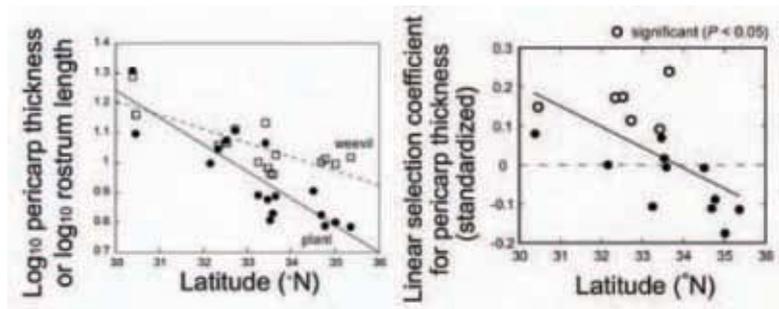


Fig. 4. Latitudinal gradients of coevolving trait size (left) and natural selection strength exerting on camellia pericarp thickness by weevils (right). After Toju & Sota (2006).

Showing findings on the geographically differentiated coevolutionary processes, I will talk about potential ecological factors triggering arms races and the consequences of coevolutionary escalation.

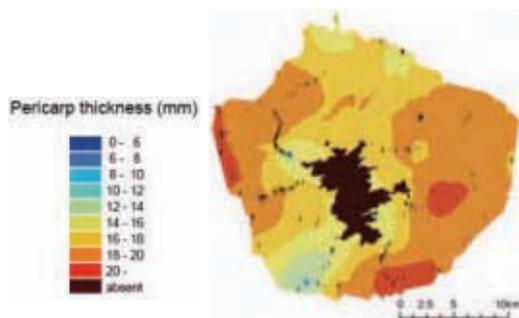


Fig. 5. Variation in camellia pericarp thickness on Yakushima Island, which suggests fine-scale spatial differentiation of the coevolutionary processes. After Toju (2008).

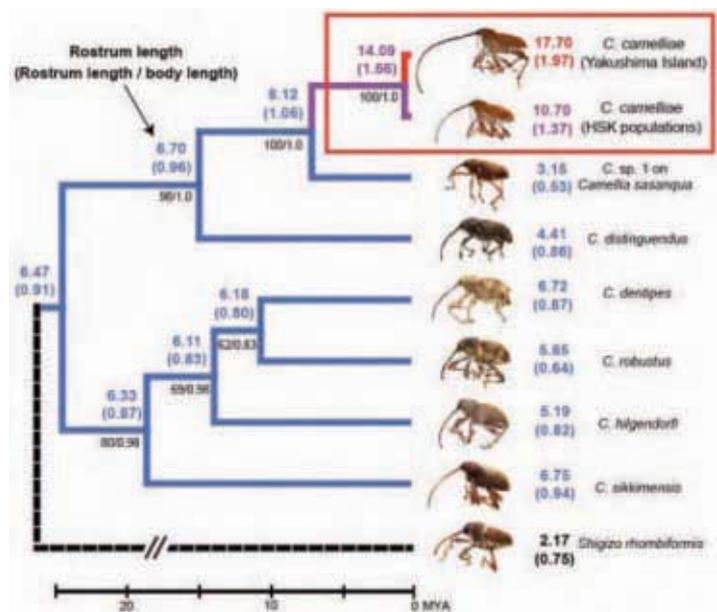


Fig. 6. Phylogeny of *Curculio* weevils suggests the rapid adaptive divergence of rostra among camellia weevil populations due to an arms race with the Japanese camellia. After Toju & Sota (2009).

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# **Right-Handed Snakes and Left-Handed Snails: Asynchronous Coevolution Drives Single-Gene Speciation**

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Tohoku University

The origin of species generally remains obscure because one can rarely identify both the genetic basis and evolutionary forces that initiated speciation. A gene for left-right reversal may generate a new species in snails, because of genital and behavioral mismatches between dextral (clockwise-coiled) and sinistral (counterclockwise-coiled) snails. However, this speciation process would be suppressed by a mating disadvantage for the reversal. Thus, the origins of reversed snails remain “the mystery of mysteries”. Here I show that sinistral snails repeatedly speciate as a coevolutionary response to specialized snake predation of the vast majority of dextrals.

Southeast Asian snakes in the family Pareatidae have been regarded as dietary specialists of terrestrial snails and slugs (figure 1). Most pareatid snakes exhibit outstanding asymmetry in mandible tooth number (figure 2), which facilitates feeding on dextral snails. Experiments directly demonstrate that sinistral snails survive snake predation superiorly to dextrals. Biogeography reveals that sinistral snail species have recurrently evolved, where the snakes continue to prey on the remaining dextral majority of snails (figure 3).

This study illuminates the crucial role of coevolutionary dynamics in speciation and exemplifies speciation by a “magic trait” that gives rise to both adaptation and reproductive isolation simultaneously at a single locus for left-right reversal. The magic trait in theory is no longer magic in real world.



Figure 1 The foraging sequence of a Japanese paratid snake, *Pareas iwasakii*, on a dextral snail. *Pareas iwasakii* approaches a snail from behind following the snail's mucus track, tilts the head leftward, grabs the basal foot near the aperture and swallows by pulling the snail body out of the shell with alternate retraction of left and right mandibles. Scale bars, 10 mm.



Figure 2 Lateral view of the left and the right mandibles with the skull of *P. iwasakii*. This specimen (KUZ 28134) shows 16 left teeth and 24 right teeth, stained with alizarin red.

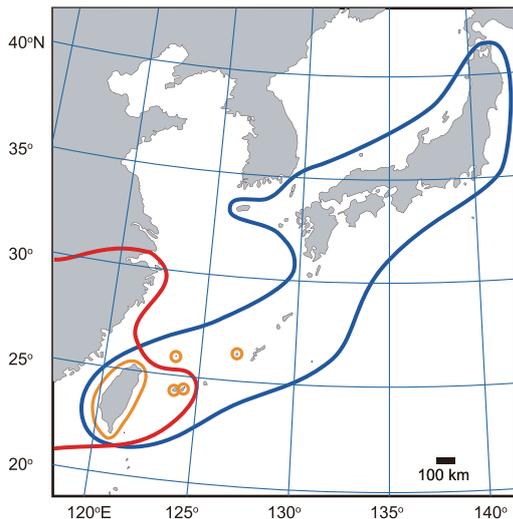


Figure 3 Geographical distributions of snails of the genus *Satsuma* (blue) and snakes of the family Pareas (red) in East Asia. Orange circles indicate islands where sinistral *Satsuma* spp. occur.

# Horizontal gene transfer and the humble origins of the world's largest flowers, Rafflesiaceae.

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**Holoparasitic Rafflesiaceae possess the world's largest flowers (up to 1 meter in diameter), and have been recently shown to have been derived from within Euphorbiaceae, the spurge family. Most euphorbs produce minute flowers, suggesting that the enormous flowers of Rafflesiaceae evolved from ancestors with tiny flowers. Recent evidence using comparative methods supports the conclusion that floral gigantism within Rafflesiaceae occurred rapidly, making this one of the most dramatic cases of size evolution reported for eukaryotes. The first goal of my talk seeks to describe this discovery and to examine why floral size increase may have been so rapid within the group. In addition, Rafflesiaceae appears to be especially prone to horizontal gene transfer, which is likely facilitated by the intimate association between parasites and their hosts. Phylogenetic relationships of gene trees from several studies within this group are strikingly different from expected species tree relationships, i.e., some DNA sequence data obtained from Rafflesiaceae is placed phylogenetically very near to their obligate hosts rather than with their closest relatives. The second goal of my talk seeks to explore these patterns of horizontal gene transfer in Rafflesiaceae.**

Over 180 years ago a remarkable plant species was discovered in the Sumatran rain forest. It was a leafless, stemless, non-photosynthetic parasite embedded in its host plant (i.e., an endophytic holoparasite) with flowers that measured nearly a meter in diameter. The plant was described as *Rafflesia arnoldii* R. Br. in honor of its co-discoverers, and crowned with the title of world's largest flower (Figure 1). Almost two centuries after this discovery, surprisingly little is known about the evolution of the extraordinary reproductive morphology of this species and its closest large-flowered relatives.



**Figure 1.** An onlooker gazes deep into the blossom of the world's largest flower, *Rafflesia arnoldii* R. Br. (Photograph courtesy of J. Holden)

*Placing holoparasites in the angiosperm Tree of Life.* A major factor contributing to our lack of understanding has been the difficulty of pinpointing the closest relatives of holoparasitic angiosperms. Despite remarkable transformations in our knowledge of plant phylogenetic relationships during the past thirty years, especially in light of the wealth of new DNA sequence data, our understanding of holoparasitic plant relationships has remained relatively static. This is largely due to their reduced vegetative morphology, highly modified reproductive structures, and anomalous molecular evolution, which has made them difficult to compare to other free-living putative relatives. Fortunately, the use of mitochondrial and low-copy nuclear genes, combined with model-based phylogenetic approaches using maximum likelihood and Bayesian inference, is providing insights on their evolutionary relationships.

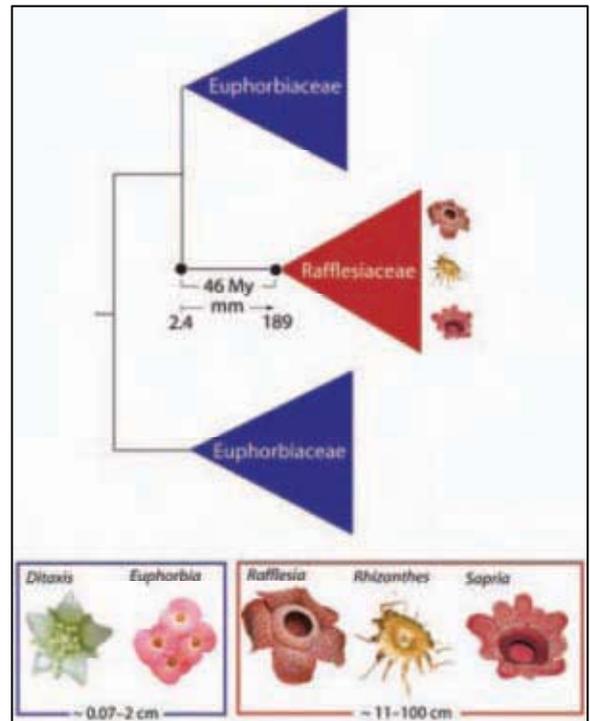
*Are most holoparasitic angiosperms monophyletic?* Traditional classifications based on morphology have tended to include the majority of holoparasitic angiosperms as members of Rafflesiaceae in the broadest sense (sometimes termed Rafflesiales), implying a single evolutionary origin of these species. This grouping was based principally on their shared reduced parasitic vegetative bodies, and included both enormous and tiny flowered species. Molecular phylogenetic studies conducted in part by our lab, supported by fresh morphological reassessments, however, have overturned this argument by showing that these genera belong to several distantly related groups. Rafflesiaceae today are restricted to some of the largest-flowered members of the plant kingdom, and exhibit remarkable variation in flower diameter spanning nearly an order of magnitude (~11–100 cm). Several recent studies have placed Rafflesiaceae *sensu stricto* as a member of Malpighiales, which is one of the most diverse flowering plant lineages. The group encompasses more than 16,000 species spanning tremendous morphological and ecological diversity, and includes numerous economically important species, such as cassava, flax, poinsettia, poplar, and the rubber tree. Our lab recently

resolved the problem of determining the relationships of Rafflesiaceae within Malpighiales by sequencing numerous gene regions from dozens of their closest Malpighialeans relatives. These efforts support the surprising finding that Rafflesiaceae are embedded within the spurge family (Euphorbiaceae), whose flowers measure only a few millimeters in diameter (Figure 2).

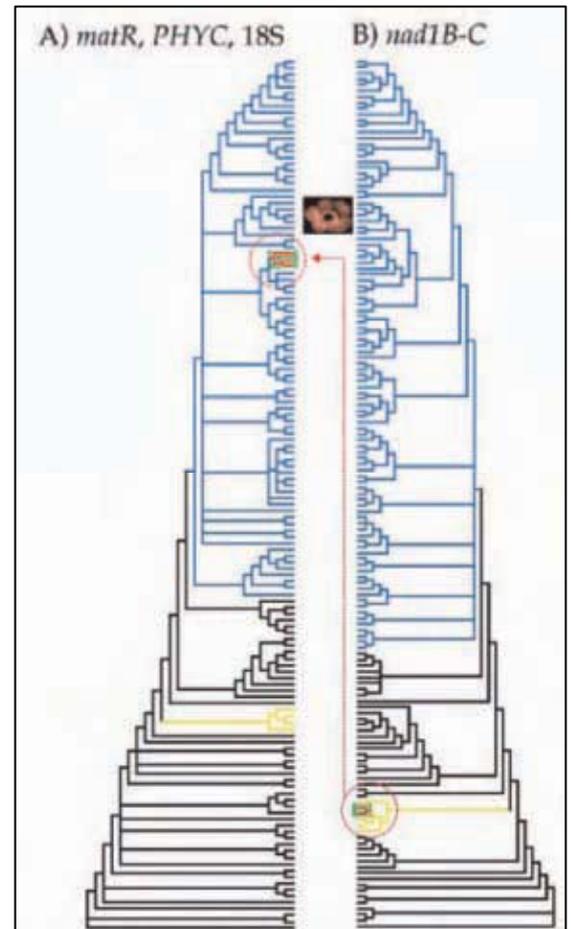
*The humble origins of the world's largest flowers.* We combined this detailed phylogenetic information and data on flower size with sophisticated comparative analyses of size evolution to determine the tempo of size evolution in these species for the first time. Our estimates indicated that Rafflesiaceae evolved from their tiny-flowered ancestors in a burst of floral gigantism at a rate that was 91 times faster on the stem lineage of Rafflesiaceae than compared with their closest relatives. The change in flower size along this stem lineage increased from ~2.4 to 189 mm at a healthy rate of ~10% per million years, under the most conservative estimates. Furthermore, since it is likely that the period of accelerated size evolution was restricted to a smaller portion of this lineage's ~46-million year duration, the rate of floral size increase may have been dramatically higher. I will discuss some likely explanations as to why floral size increase may have been so rapid in this group.

*Rafflesiaceae and studies of horizontal gene transfer.* In addition to our exploration of floral evolution in Rafflesiaceae we are also using this clade to study the dynamics of horizontal gene transfer in a plant parasitic system. It has recently been shown that plant mitochondrial genomes undergo surprisingly frequent and widespread horizontal gene transfers (HGTs). Mechanistic explanations for how gene transfers occur, however, have been speculative. Our work on Rafflesiaceae has provided the first clear insights into this phenomenon. We have demonstrated evidence of gene transfer in Rafflesiaceae by using phylogenetic approaches (Figure 3). Thus, some HGTs are facilitated by the intimate association between a plant parasite and its host. For example, certain gene or intron regions from Rafflesiaceae are placed phylogenetically very near to their obligate hosts rather than with their Malpighialeans relatives, and are inferred to have been acquired via host-to-parasite gene transfer. I will discuss our work in progress on HGT biology in Rafflesiaceae, which is focused on establishing the magnitude of gene transfer and the functionality of transgenes in their recipient species using transcriptomic and phylogenomic approaches. Insights gleaned using this approach will likely help to further improve our understanding of the role of HGT in the evolution of novelty in the Eukaryotic Tree of Life.

**Figure 3.** Host-to-parasite gene transfer. Two conflicting hypotheses for the phylogenetic placement of Rafflesiaceae. **(A)** The strict consensus of 136 angiosperms for combined mt *matR* and nuclear (*PHYC* and ribosomal 18S) data showing a well-supported (100% BP) Malpighiales clade (in blue), plus Rafflesiaceae (in red). **(B)** The strict consensus of 147 angiosperms for mt *nad1B-C* showing a well-supported (100% BP) Malpighiales clade, which includes all members of the order except Rafflesiaceae. Rafflesiaceae are instead strongly placed (100% BP) in the basal eudicot family Vitaceae (in yellow) as sister to their host genus, *Tetrastigma*. Dashed line is hypothesized host-parasite horizontal gene transfer.



**Figure 2.** Phylogenetic placement of the world's largest flowers (Rafflesiaceae; red, also pictured bottom right) within the tiny-flowered spurges, Euphorbiaceae (blue, also pictured bottom left). Floral diameter size increase along the 46 million year (Myr) stem lineage of Rafflesiaceae resulted from very high rates of floral evolution.



# Secondary endosymbioses as a driving force of diversification of algae.

Isao Inouye

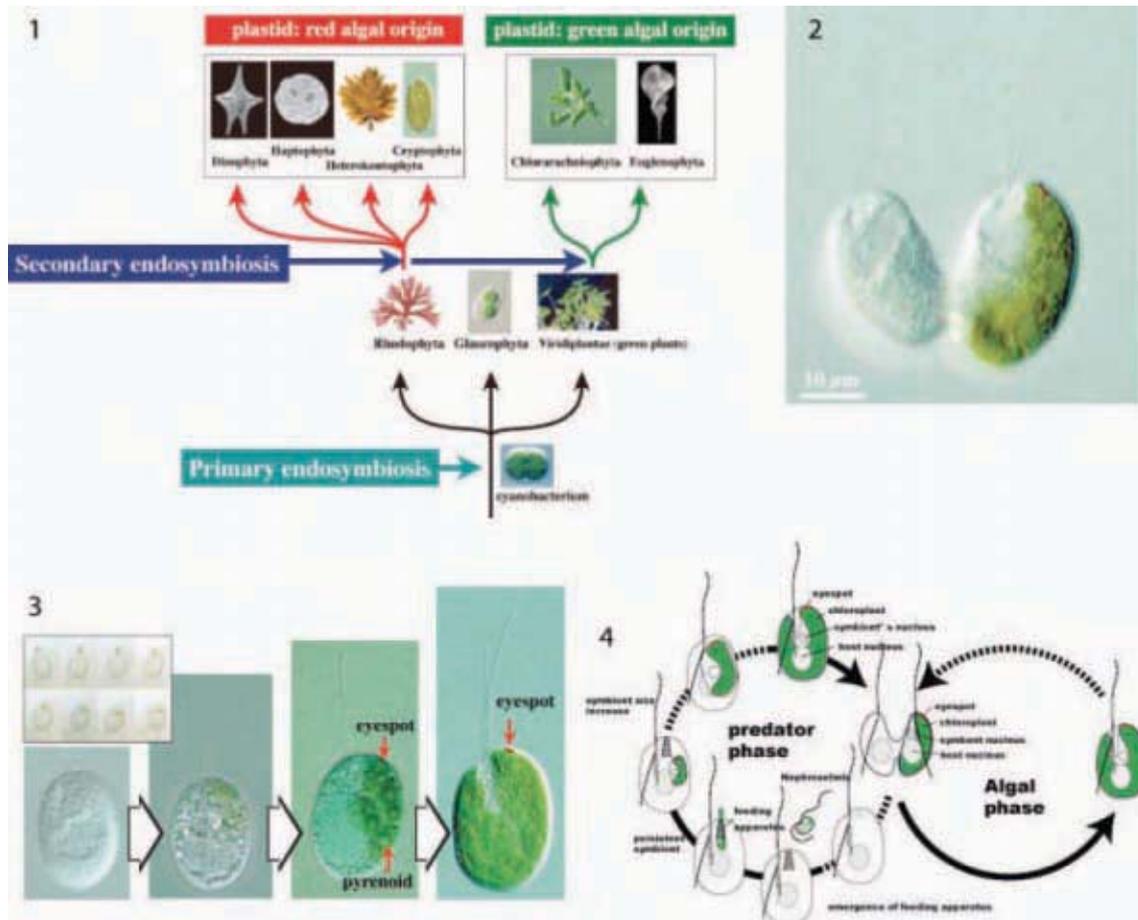
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Algae, generally defined as oxygenic photosynthetic organisms inhabiting aquatic environment, are a major component of primary producers on the present-day earth. It comprises prokaryotic cyanobacteria (blue green algae) and nine eukaryotic autotrophic groups, including the terrestrial plants (land plants) as a derivative from the green algal lineage. Eukaryotic algae originated from an evolutionary event in which phagotrophic eukaryote engulfed a cyanobacterium and integrated it as a cell component, a plastid. This event is called primary endosymbiosis, and the descendants of the first eukaryotic alga are referred to as the primary plants. The glaucophytes, rhodophytes and green plants are believed to be members of the primary plants. However, there are many other eukaryotic algae contributing for primary production on the present-day earth, including heterokontophytes (brown algae, diatoms, etc.), euglenophytes, dinoflagellates, haptophytes, cryptophytes and chlorarachniophytes. These algal groups are positioned in various supergroups of eukaryotes that have been revealed by recent molecular phylogenetic analyses, indicating that these algae are phylogenetically distinct from the supergroup Plantae comprised of aforementioned three primary plants. They have obviously acquired plastids via different evolutionary events.

It is understood that two third of eukaryotic algae acquired plastids via engulfment of primary plants. This evolutionary event, known as secondary endosymbiosis, occurred more than once. The endosymbioses with the green algae produced the euglenophytes and chlorarachniophytes, and endosymbioses with red algae produced the cryptophytes, dinoflagellates, haptophytes and even a malaria parasite *Plasmodium*. The Cryptophyta and Chlorarachniophyta, which still possess vestigial nuclei of eukaryotic endosymbionts, are the living evidences of the secondary endosymbioses, and recent molecular phylogeny have succeeded to paint a global picture of diversification of algae via secondary endosymbioses.

The detailed process of the secondary endosymbiosis, especially its early stage, is still unclear. Various examples proceeding secondary or tertiary endosymbioses allow considering what are happening in the process of plastid acquisition. *Hatena arenicola*, a flagellate belonging to the Kathabrepharida and possesses a green algal endosymbiont, is a unique example to consider early evolutionary stage of plastid acquisition. In *H. arenicola*, symbiont's plastid is selectively enlarged, while other organelles are degraded or completely lost, and that the eyespot of the symbiont is always positioned at the cell apex of the host cell. These facts indicate that there are functional associations between *H. arenicola* and green algal symbiont. The cell division of *H. arenicola* is unusual. The symbiont is inherited only to one daughter cell, resulting in one colorless (white) cell and one symbiont-harboring (green) cell. Without exception, the symbiont is inherited to the right (when viewed from the dorsal side of the host cell) daughter cell, suggesting that *H. arenicola* controls the inheritance of the symbiont. In the white cell, a complex feeding apparatus known in other kathablepharids appears at the cell apex that corresponds to the location of the eyespot in green cells. White cells engulf free-living green alga (*Nephroselmis*, Nephroselmidophyceae, a prasinophyte alga in the former sense) from outside and return to green cells. This is considered as an example of an early stage of the secondary endosymbiosis. Host and symbiont seem to be functionally integrated to a great extent except synchronization of cell divisions of the host and symbiont. They are still two independent organisms but seem to be at the stage immediately before to be a true plant. Based on the case of *H. arenicola* and other examples of secondary or tertiary endosymbioses, we could discuss evolutionary processes of the secondary endosymbioses that proceed enslavement of eukaryotic algae and eventually integrate them as plastids.

Fig. 1. Evolution and diversification of algae via two types of endosymbioses. 2. *Hatena arenicola* at final stage of cell division, resulting in one green and one white cell. 3. A white cell feeding a green flagellate *Nephroselmis* (left top). Various transition stages from white cell to green cell. 4. Proposed life cycle of *Hatena arenicola* comprised of plant-like life algal phase and animal-like predator phase.



# Lichens: evolutionary relationship between lichenized fungi and algae.

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Lichen is a symbiotic organism consisted of fungus and alga (and/or cyanobacterium). As the result of symbiosis, it generally produces a special structure of thallus and secondary chemical substances, and survives in severe environments. Genus *Trebouxia*, one of the common algal photobionts, is usually not found as a free-living colony state in a field and hypothesized to be evolved as a lichen component. Because of these intimate relationships between mycobiont and photobiont, “co-evolution” in lichen has been widely discussed among researchers (Ahmadjian 1987). However, in less than 2% of lichens has the photobiont ever been identified at the species level, and fairly often not even the generic affiliation is known (Honegger 2008). In this presentation, evolutionary selection process of lichen photobiont will be discussed based on the studies of genetic diversity in the symbionts of *Parmotrema tinctorum* (Parmeliaceae, Ascomycota) (Fig. 1).



Fig. 1. *Parmotrema tinctorum*.

*Parmotrema tinctorum* vegetatively reproduces by its diaspores called isidia that contain both mycobiont and photobiont. Although the same combination of symbionts was expected to be detected in a local population, the high genetic diversity of photobiont was detected based on ITS rDNA in the population of Shimizu District (Fig. 2). Interestingly, the high genetic diversity was detected in rural area and it is dramatically compared to the low genetic diversity of photobionts in urban area (collection site number 9, 17-24, 26). To test if air pollution contributes the low genetic diversity in urban area, Tajima’s D test was performed with respect to the air pollution level of NO<sub>2</sub> within the investigated area. Our results showed the genetic status of populations in polluted area are under selection ( $D < 0$ ), suggesting that the only favorable individuals that have their compatible photobionts are selected to survive in the urban environment.

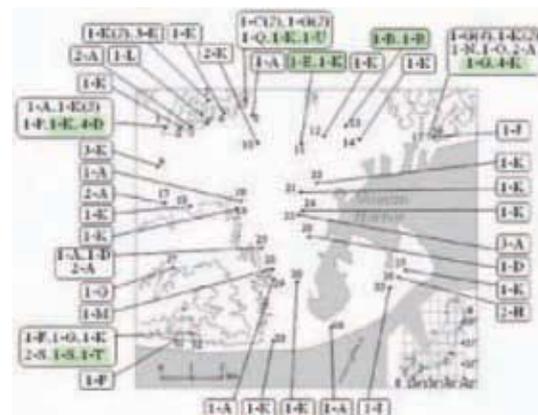


Fig. 2. Genetic diversity of *P. tinctorum* in Shimizu District. Genetic combination types of mycobiont (1-4) and photobiont (A-U) are designated and highlighted combinations are collected from the same tombstone. (Ohmura *et al.* 2006).

To further investigate the selection process of the photobiont of *P. tinctorum*, we tested the hypothesis that the genetic diversity of photobiont can be generated by photobiont exchange between the original photobiont of lichen and the compatible algal partners on its surrounding substrate. In this case, the lichen individuals can acquire their favorable algal partners in the environment and one

of the algal haplotypes on the substrate should be identical to the haplotype of lichen photobiont as the result of photobiont exchange. We employed Denature Gradient Gel Electrophoresis (DGGE) method based on *rbcL* partial sequence with samples of *P. tinctorum* and their substrate algae collected at 20 spots on tombstones in Shizuoka City. Our results detected one to five bands on each substrate and most of bands were identified as haplotypes of *Trebouxia corticola* which is the photobiont for *P. tinctorum*. Importantly, the photobiont haplotype of *P. tinctorum* was identical to one of the *T. corticola* haplotypes from the substrate in five of 20 collecting spots. As a result, these genetically identical patterns provide an evidence of photobiont exchange in vegetatively reproducing lichens.

Photobiont exchange might occur not only within the same lichen species but also among different lichen species, because the same photobiont haplotypes were also detected from other species in distantly related lichen genera (e.g., *Dirinaria*, *Physcia*, and *Pertusaria*) (Fig. 4). This result further supports that the co-evolution of lichen symbionts is not a matter of strict co-speciation, but a matter of selection to maintain the optimal symbiotic relationship between mycobiont and photobiont. The selection of photobionts by lichen-forming fungi may have been evolved independently in each phylogenetic group.

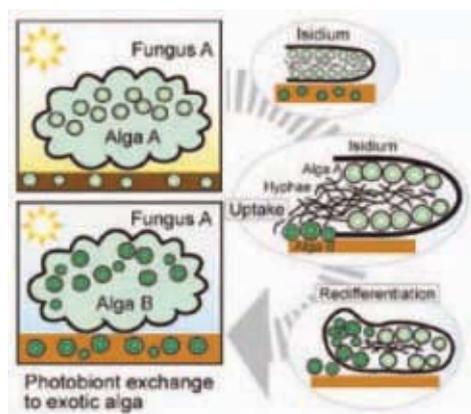


Fig. 3. Hypothesis of photobiont exchange in a vegetatively reproducing lichen.

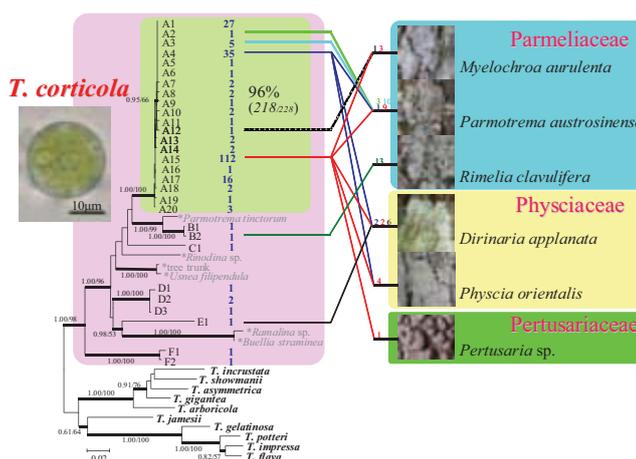


Fig. 4. Phylogeny of lichen photobionts between *Parmotrema tinctorum* and other lichens.

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# **Evolutionary relationships between powdery mildew fungi, obligate biotroph of plants, and their host plants**

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The Erysiphaceae (Ascomycete: Erysiphales) are a group of obligately parasitic fungi of plants, which cause powdery mildew diseases on about 10,000 angiosperm species. With the exception of the dormant stage, their life cycle completely depends on living hosts, from which they obtain nutrients without killing the host cells and without which they are unable to survive. To maintain the obligate parasitic life cycle, Erysiphaceae have developed highly specific and sophisticated mechanisms to avoid the resistance system of the host, to obtain nutrient resources from the host without injuring the host cells, to synchronize their life-cycle parameters to those of the host, etc. As a natural outcome, most species of the Erysiphaceae show strict host specificity, in which a given species or race can infect and utilize a narrow range of host plants, or sometimes only a particular species of host. It is therefore expected that the association between the Erysiphaceae and their hosts would have been conserved during the course of their evolution. If fungal lineages remain associated with their hosts over a long time, events that isolate the host populations may also isolate the populations of their associated fungi, which may eventually result in co-speciation of the parasites and their hosts. In this context, it has been suggested that the phylogeny of the Erysiphaceae is concordant with that of its host plants. There is, however, no obvious evidence to indicate co-speciation between Erysiphaceae and their host plants. In this presentation, evolutionary relationships between powdery mildew fungi and their host plants will be discussed based on molecular phylogeny using nuclear rDNA sequences.

## **1. Morphology of ascomatal appendages is a result of adaptive evolution to plants**

Hirata (1976) pointed out that the Erysiphaceae are divided into two distinct groups of genera based on their host ranges, i.e., tree-parasitic genera and herb-parasitic genera. All of the herb-parasitic genera have mycelioid appendages on ascomata, whereas deciduous tree-parasitic genera represent various types of appendages such as dichotomously branched, uncinata to circinate, clavate, or bristle-like (Fig. 1). The ascomata are considered an organ that endures the winter season in the Erysiphaceae. Ascomata of deciduous trees are easily dislodged and blown off the leaf surface by wind or rain after maturing, where upon they adhere to the bark of twigs by the appendages, and function as primary infection sources for the next year. On the contrary, ascomata of herbaceous- and evergreen tree-parasitic genera remain on the leaf surface even after maturing. Thus, the overwintering behavior of ascomata differs markedly between herbaceous and evergreen tree-parasitic genera and deciduous tree-parasitic genera with the morphology of appendages considered to be a result of adaptation by ascomata for overwintering. Molecular analyses show that the genera having mycelioid appendages are polyphyletic. This may indicate that the mycelioid appendages are derived characters as a result of simplification of

appendages that occurred multiple times as an adaptation to herb-parasitism.

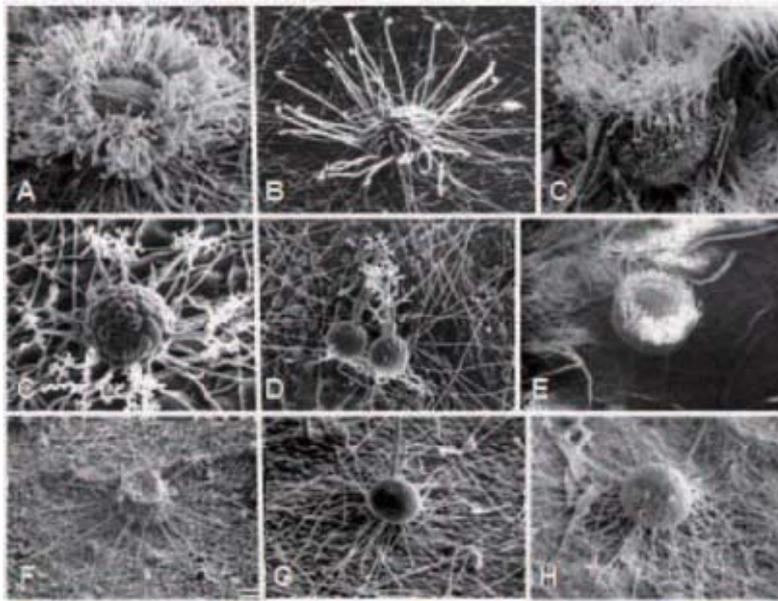


Fig. 1. Ascomata (chasmothecia) of the Erysiphaceae with various types of appendages.

A,B,C appendages with uncinately or circinate tips; C,D appendages with dichotomously branching tips; E appendages with clavate shape; F bristle-like appendages with bulbous base; G,H mycelioid appendages.

A *Sawadaea*; B *Erysiphe* sect. *Uncinula*; C *Pleochaeta*; D *Erysiphe* sect. *Microsphaera*; E *Podosphaera* sect. *Podosphaera*; F *Phyllactinia*; G *Erysiphe* sect. *Erysiphe*; H *Neoerysiphe*

## 2. Relationships between host specialization and genetic divergence

To evaluate the relationship between phylogeny and infectivity, we compared fungal groupings by phylogenetic analysis of the ITS region with groupings from the cross-inoculation tests by Abiko (1978, 1982a, 1982b). The groupings based on the molecular phylogeny were consistent with the groupings based on infectivity with an exception. The results show a close relationship between fungal phylogeny inferred from nucleotide sequences and host specialization. Since the isolates that Abiko used in his reports were not available, we used powdery mildew isolates different from the Abiko's isolates in the phylogenetic analysis. It is noteworthy that the two different experiments were carried out using different isolates at different times, but revealed quite similar groups of fungi. This may indicate that niche separation caused by host specialization triggers genetic divergence in this group of fungi.

## 3. Co-speciation or host-jumping?

Comparative molecular phylogeny of powdery mildews and their host plants did not show strict host-parasite co-speciation in most groups of Erysiphaceae. However, close relationships were detected between the genus *Golovinomyces* and their asteraceous hosts, suggesting host-parasite co-speciation. The host-parasite relationships were not so strict in the genus *Leveillula* and subsection *Magnicellulatae*, both of which have Asteraceae as main host family like *Golovinomyces*. The causal factors of these results will be discussed from the aspect of evolutionary timing of these powdery mildews and plants.

# Fungal Diversity and Distribution: Challenges for Evolutionary Biologists and Conservation Scientists

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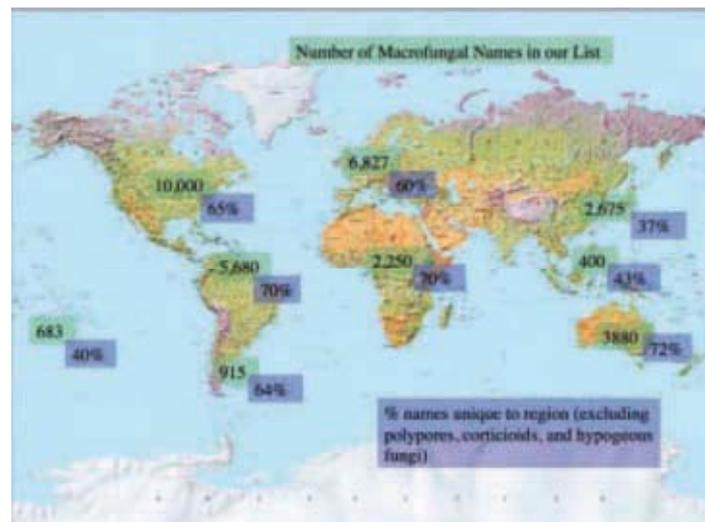
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Fungi are among the most important organisms in the world, not only because of their vital roles in ecosystem functions, but also because of their influence on humans and human-related activities (Mueller and Bills 2004). However, only limited and incomplete information is currently available for most species and current estimates of species numbers for fungi differ significantly. This lack of basic information on taxonomic diversity has significant implications for many aspects of evolutionary biology, e.g., phylogenetic hypotheses, the role that biodiversity plays in providing resilience to perturbations, coevolutionary relationships and processes, interpretations of biogeographic patterns, natural products screening programs, etc. Having a stable and accepted estimate of taxonomic diversity for fungi is also necessary to enable fungi to be included in considerations of biodiversity conservation, land-use planning and management, and related subjects. While the figure of 1.5 million estimated fungal species is commonly used, critics have questioned the validity of this estimate. Data on biogeographic distributions, levels of endemism, and host specificity must be taken into account when developing estimates of global fungal diversity.

The results of three different types of studies will be discussed as they relate to the question of fungal diversity.

(1) A metadata analysis of diversity and distribution data for macrofungi and trees from a compilation of published and unpublished studies of plot-based studies (Schmit et al. 2005). We found that macrofungi are neither more nor less widely distributed than trees, and tree species richness was a good surrogate for macrofungal species richness, even at sites with high tree richness (this does not work in areas without trees such as subarctic meadows, tundra, and steppe). However, while plant diversity may be a good indicator of fungal diversity, sites with similar tree species may not hold similar fungal species. Thus, plant distributions cannot be used as a surrogate for fungal distributions.



(2) A compilation of species lists from various regions of the world plus estimations of

the number of unknown fungi by collaborating experts to estimate global species diversity for various groups of fungi (Mueller and Schmit 2007, Mueller et al. 2007, Schmit and Mueller 2007). We conservatively estimate that there is a minimum of 712,000 extant fungal species worldwide, but we recognize that the actual species richness is likely much higher.

(3) Rigorous phylogeographic studies of various macrofungi (Wu et al. 2000; Mueller and Hosaka, in prep). Numerous undescribed species have been uncovered and most species have discrete distribution patterns.

The results from each of these different studies are consistent with the hypothesis that fungi are a megadiverse group. Additionally, these studies provided complementary results for understanding the evolution, distribution, and ecology of macrofungi.

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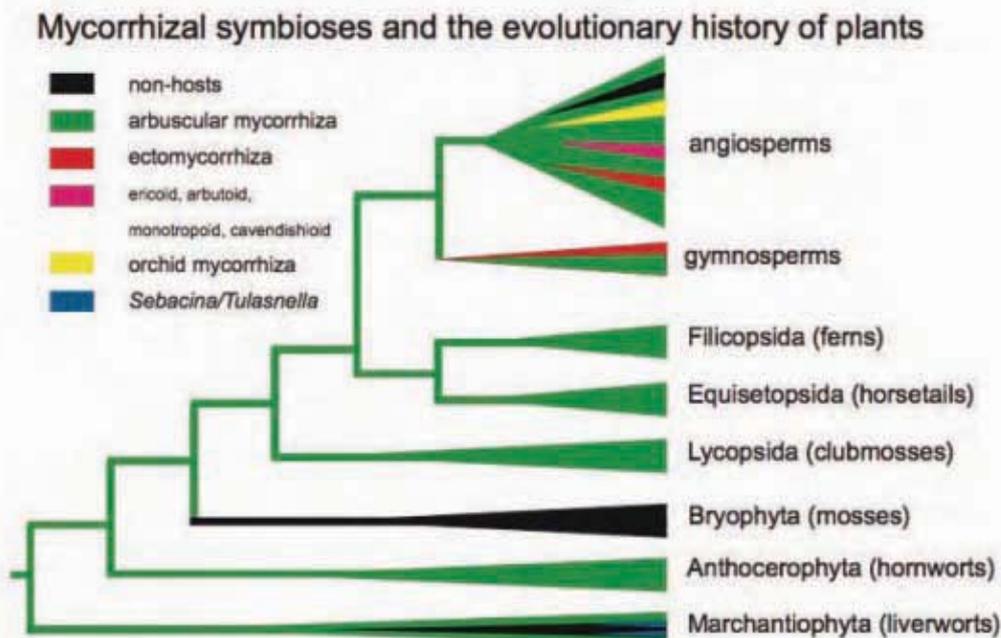
# The evolutionary history of arbuscular mycorrhiza

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The great majority of land plants form arbuscular mycorrhiza (AM), a mutualistic symbiosis, with fungi from the phylum Glomeromycota. As glomeromycotan fungi have been found as fossils from the Ordovician (460 million years ago), when plants were most likely in a very early stage of their evolution, it was hypothesized that this association might have been instrumental in the successful conquest of the land masses by green plants (Redecker et al. 2000). This view is supported by the presence of this symbiosis in most basal land plant lineages. In fact, arbuscules, the symbiotic structures, were demonstrated as early as the Devonian (400 million years ago



(Remy et al. 1994)). However, based on molecular clock analyses, some authors placed the origin of both land plants and fungi much earlier (Heckman et al. 2001).

AM fungi were previously placed in the Zygomycota, a paraphyletic assemblage of diverse fungal groups, before a separate phylum was established for them (Schüßler et al. 2001). Ribosomal gene phylogenies placed the Glomeromycota as sister group to "higher fungi" (i.e. Asco- and Basidiomycota), which interestingly is not the case with phylogenies of protein-coding genes (Lee and Young 2009; Redecker and Raab 2006)

In the phylum Glomeromycota, only one species is known to form a different type of symbiosis: *Geosiphon pyriformis* harbours endosymbiotic cyanobacteria (Schüßler et al. 1994). Whether this association represents an ancestral type to mycorrhiza formers is still unclear.

AM obviously constitutes the ancestral type of mycorrhizal association, but many lineages of land plants later either became non-hosts or switched to different types of mycorrhiza, involving different groups of fungal symbionts, e.g. ectomycorrhiza or orchid mycorrhiza.

Diversity of plants and AM fungi seem to influence each other (van der Heijden et al. 1998), but

the number of host plant species is orders of magnitude higher than the mere 200 morphospecies that have been described in the Glomeromycota. The morphology of these fungi apparently has been remarkably stable over long periods of time, but the paucity of microscopically-distinguishable characters of their thallus may have contributed to underestimate the true diversity of this fungal group. Host specificity is generally relatively low in AM, with many fungi and plant species associating with each other (Klironomos 2000), therefore the potential for co-speciation seems to be low. Molecular studies have shown that nevertheless there are preferences between the hosts (Helgason et al. 2002; Sykorová et al. 2007), but host specificity in the sense of a narrowed range of associated symbionts has only been shown in mycoheterotrophic plants, i.e. achlorophyllous parasites of the mycorrhizal network (Bidartondo et al. 2002). Generally the evolutionary pressure to remain compatible with a wide range of hosts may have slowed down diversification. On the plant side there may be a trade-off between the advantage of having an exclusive, specialized fungal symbiont and the ability to plug into a universal network that also includes other plant species.

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# Common mycorrhizal networks and their significance to land plant diversification. Achlorophyllous plants as part of the mycorrhizal networks.

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In the typical mycorrhizal symbiosis, which links soil fungi with the roots of ~90% of plant species, fungi exploit soil mineral nutrients in return for plant produced carbon. In contrast, mycoheterotrophy, in which plants instead receive carbon from their associated fungi, has been found in several forest-understorey achlorophyllous plants from various families, including orchids. In temperate ecosystems, orchids have highly specific association to narrow fungal taxa that, in turn, form mycorrhizae with surrounding green plants. Isotopic methods support that the surrounding plants are the ultimate carbon source for the orchid-fungi symbiosis. This nicely illustrates functional outcomes of shared mycorrhizal networks among plants, resulting from a low specificity of most fungal associates.

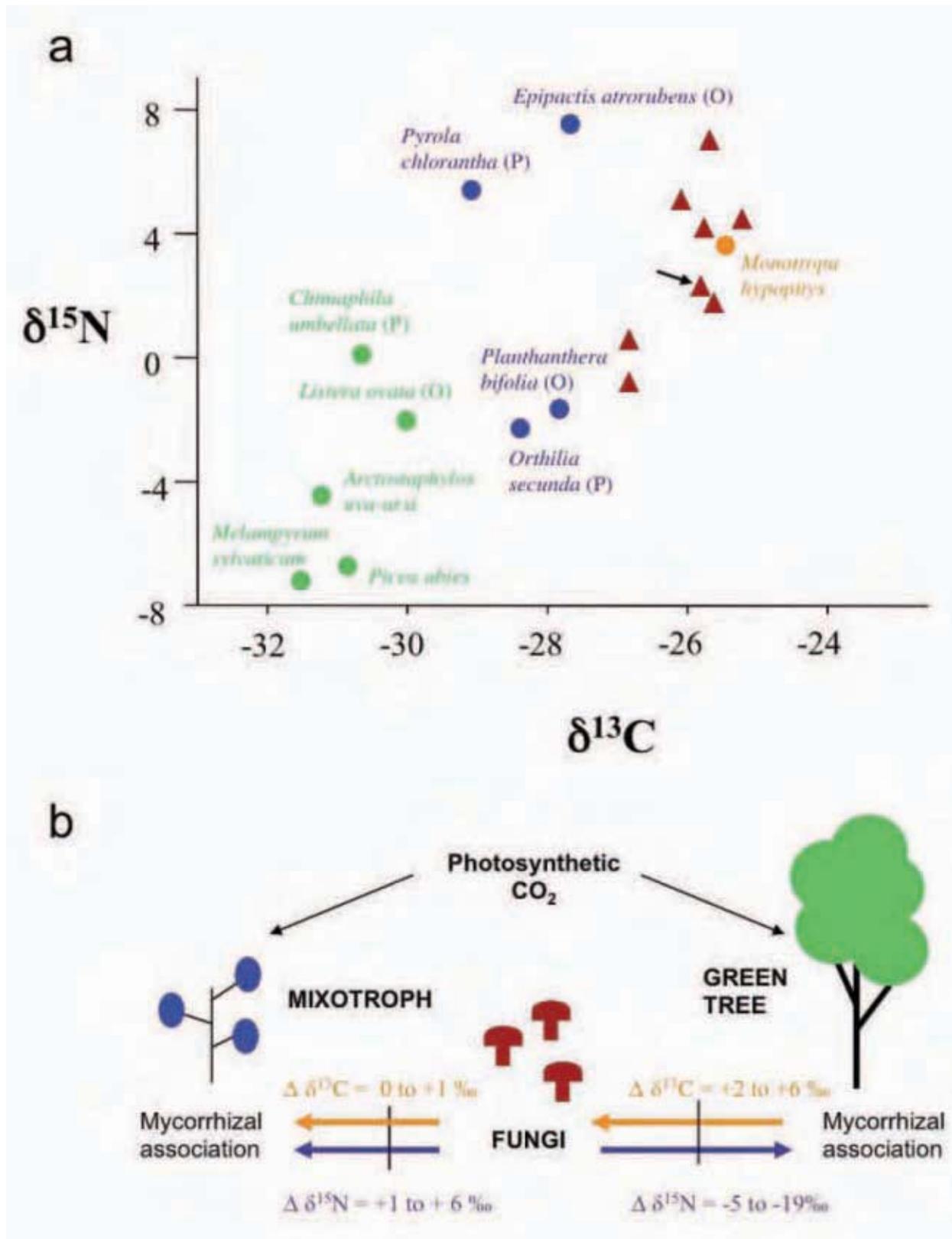
When we investigated tropical mycoheterotrophic orchids, we found a more flexible picture: some orchids were not specific, such as *Aphyllorchis* spp. from Thailand; some associated with normally saprobic fungi (*Mycena*, *Gymnopus*, *Resinicium*), such as *Wullschlaegelia* and *Gastrodia* spp. from Caribbean and Mascarene Islands, with evidence that decaying plant material is their ultimate carbon source. Based on this, we revisited the current understanding of evolution of mycoheterotrophic clades and their adaptation to tropical *versus* temperate conditions.

Moreover, green forest-understorey plants, phylogenetically close to mycoheterotrophs, were recently shown to be mixotrophic, i.e. they receive carbon from both their fungi and photosynthesis. Phylogenetic analyses suggest that in orchids and pyroloids (*Ericaceae*), mixotrophy preceded the evolution of mycoheterotrophy. In some mixotrophic *Cephalanthera* and *Epipactis* spp., achlorophyllous plants (AP) can even be found rarely in natural populations. To understand AP rarity, we investigated *in situ* AP morphology, physiology (gas exchanges) and fitness over several years, in several European populations. We showed that impaired carbon budget and bad regulation of water exchanges due to vestigial leaves and stomata, made AP unfit. Thus, we propose, *a contrario*, that co-evolution of several traits is required for successful transition to mycoheterotrophy, explaining why this transition remains rare in mixotrophic lineages.

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**Figure** (next page): Stable isotope abundances in mixotrophic and mycoheterotrophic plants linked to a mycorrhizal network. Isotope abundance is expressed in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (in ‰) relative to international standards. **(a)** Stable isotope abundances in plants and mycorrhizal fungi from a boreal Estonian forest (Tedersoo *et al.*, 2007). Species include orchids (O) and pyroloids (P), a tribe of Ericaceae. Autotrophic plants at this site: *Arctostaphylos uva-ursi*, *Listera ovata*, *Melampyrum sylvaticum*, *Picea abies*. Mixotrophic plants: *Epipactis atrorubens*, *Platanthera bifolia*, *Orthilia secunda* and *Pyrola chlorantha*. *Monotropa hypopitys* is mycoheterotrophic and triangles represent ectomycorrhizal fungi – the one indicated by an arrow (*Tricholoma myomyces*) is mycorrhizal on *M. hypopitys*. **(b)** A summary of C and N nutrient flow in mixotrophic plants and associated plants and fungi, with ranges of isotopic fractionation at the various plant/fungus interfaces (from Selosse & Roy, 2009).



# Gut yeasts in insects: biodiversity and application

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Some of the eukaryotic endosymbionts that have been discovered have obligate roles in the lives of their insect hosts, such as detoxifying food resources, and/or providing vitamins, amino acids, lipids and sterols. Those include true yeasts (Saccharomycetes) as well as reduced yeastlike symbionts (YLS) derived from several groups of filamentous ascomycetes. Some yeasts and YLS are found in a mycetome, a blind sac for the symbionts, or in several parts of insect gut (Fig. 1).

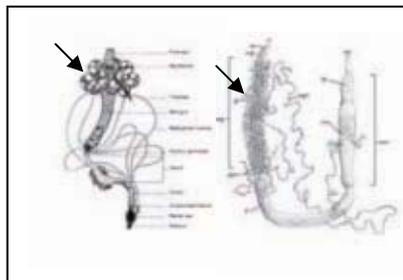


Fig. 1. Location of gut yeasts (arrows) in *Lasioderma serricorne* (Anobiidae, left) and *Megalodacne heros* (Erotylidae, right). From Milne (1963) and McHugh et al. (1997).

Over the last ten years we examined a variety of insects collected from several places in the USA and Panama, and more than 2,000 yeasts were isolated from the digestive tract of insects in this study. Biodiversity of the gut yeasts, insect-yeast relationships, and potential application of those yeasts are discussed.

## 1. Gut yeasts are diverse in yeast phylogeny.

The gut yeasts from insects were not monophyletic. The majority of the yeasts were ascomycetes (Saccharomycetes) although a few basidiomycetous yeasts related to *Cryptococcus* or *Trichosporon* were repeatedly isolated. Based on rDNA sequence analyses, the gut yeasts were grouped as more than 300 genotypes in at least 45 phylogenetic clusters distributed throughout the yeast phylogenetic tree (Fig. 2). Several previously unknown, entirely insect-associated yeast clades were discovered, such as the *Candida tanzawaensis* clade, which contained 30 % of all the gut yeasts collected from mushroom feeding beetles.

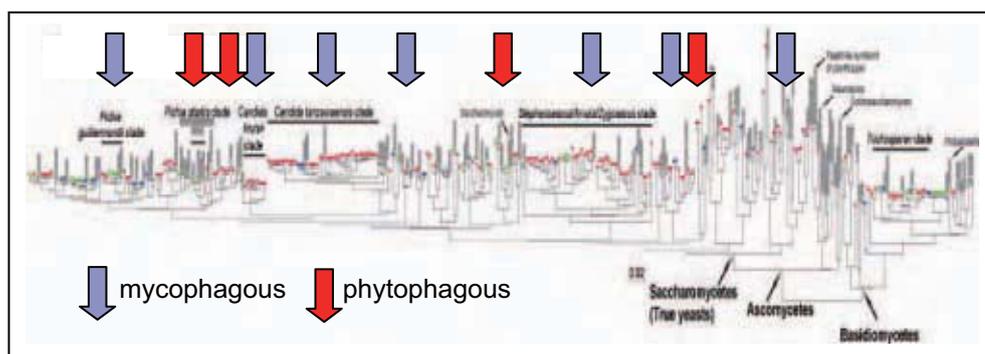


Fig. 2. A phylogenetic tree from ribosomal DNA sequences. The colored taxa on the tree represent the genotypes of gut yeasts. The blue arrows indicate the clusters of yeasts isolated from mycophagous insects and the red ones from phytophagous host insects. Based on Suh et al. (2005).

## 2. Yeasts from different host habitats are phylogenetically distinct.

Gut yeasts from mycophagous insects were phylogenetically distinct from those of phytophagous taxa (Fig.2). For example, all the gut yeasts in the *Candida tanzawaensis* clade (>200 strains in at least 16 species) and the *Candida kruisii* clade (100 strains in 10 species) were isolated from fungus-feeders while those belong to the *Candida albicans* clade (41 strains in 14 species) were only found in plant-feeding insects, such as elatrids, owlfiles,

fishflies, earwigs, and crickets. This result supports the view that the gut yeast communities could be closely related to the habitats of host insects, especially diets.

### 3. Host Specificity

Yeast-insect specificity was observed between certain yeasts and insects across broad geographical ranges and multiple developmental stages of some beetles;

- Same yeast from same insects collected from different locations
- Same yeast from same insects collected in different times
- Same yeast from different stages of insect life cycle

### 4. Insect gut is a hidden source of novel yeasts.

Based on DNA sequence analyses and other taxonomic characteristics, we found that almost 70% of the gut yeasts isolated from this study have never been described before and those yeasts could be classified as almost 200 undescribed species (Fig. 3). A Bayesian analysis of species discovery rates predicts further sampling of previously sampled habitats in one study could easily produce another 100 species. The discovery of this large number of undescribed yeasts gains greater significance with the realization that fewer than 700 species of ascomycetous yeasts have been described previously from all of the Earth's habitats (Boekhout 2005).

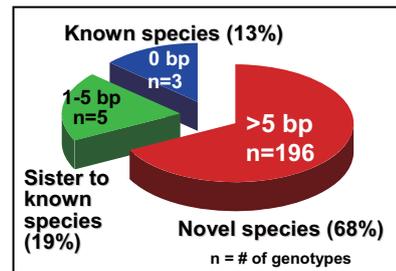


Fig. 3. Numbers of genotypes in gut yeasts from mushroom-feeding beetles based on the nucleotide differences of D1D2 region of LSU rDNA from previously described species. Two yeasts in more than 1% of difference (>5bp) in the sequence are generally considered as different species.

### 5. Culture-independent studies

By cloning of the DNAs from whole gut contents, we confirmed the presence of the yeasts isolated by culture-based methods as well as several other microorganisms, such as *Malassezia* spp., *Nosema* sp. and several flagellates in selected beetle guts, which could not be cultivated by common culture methods. From the direct observation of the gut of *Odontotaenius disjunctus* (Passalidae: Coleoptera) by light microscopy and TEM, we found that several gut microorganisms, such as bacteria, yeasts, flagellates, and other eukaryotes are partitioned within the gut habitats (Fig. 4).

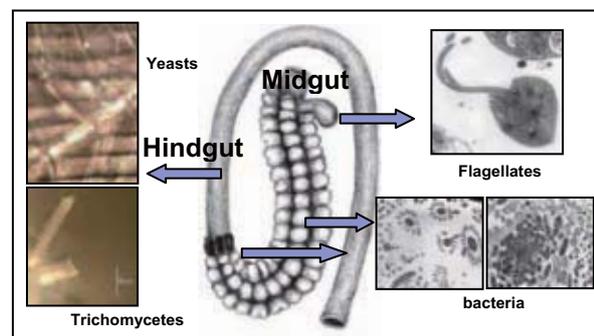


Fig. 4. Habitat Partitioning of gut microorganisms in *Odontotaenius disjunctus*. Based on Nardi et al. (2006 and unpublished) and Suh et al. (2004).

### 6. Application of gut yeasts and fungi

By understanding the roles of the gut yeasts (fungi) in host insects, these yeasts can be better applied to many industrial and scientific areas. For example, many gut microorganisms are known to help their hosts digest foods directly or indirectly. Based on our survey, more than 90% of the gut yeasts from wood-boring beetles can assimilate xylose, the major sugar of hemicellulose, and some of these can ferment xylose to produce ethanol, a potential resource for bioethanol production. More details on potential applications of the gut yeasts will be discussed.

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# The evolution of termite-egg mimicry by cuckoo fungi

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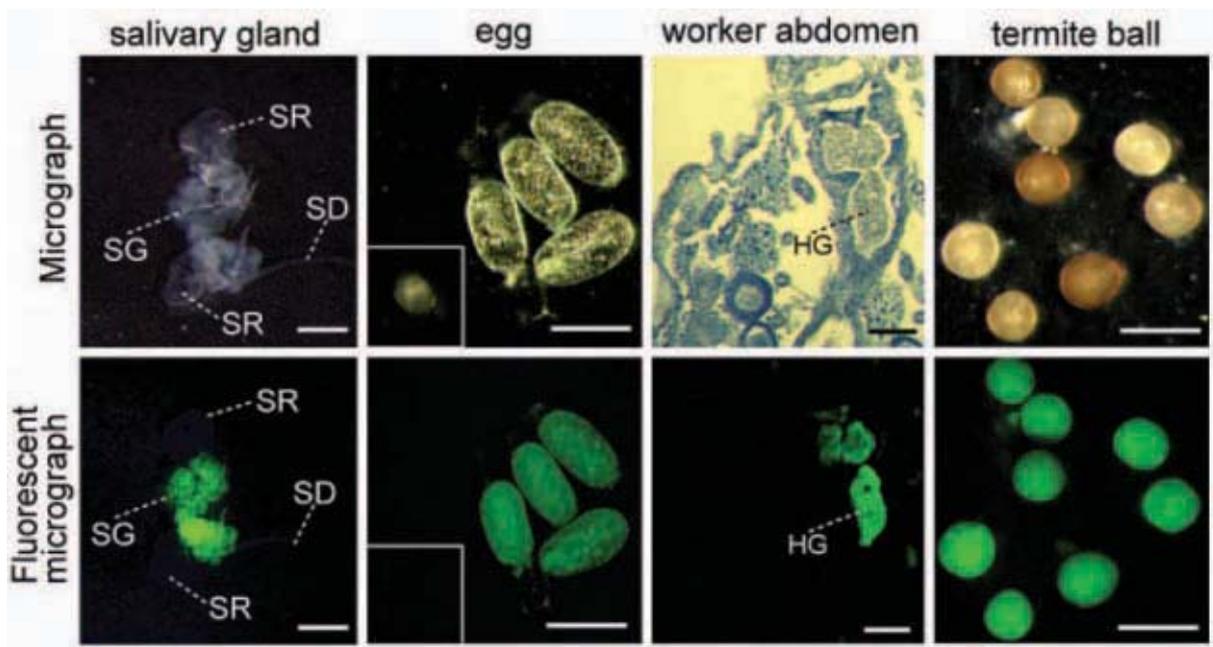
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Insects and fungi share a long history of association in various habitats, including the wood-decomposition niche. Fungal mimicry of termite eggs is one of the most striking evolutionary consequences of insect–fungus association. Termites of the genus *Reticulitermes* often harbor fungal sclerotia, called “termite balls”, along with eggs, whereby the fungus gains a competitor-free habitat in termite nests (Fig. 1). Sophisticated morphological and chemical camouflage are needed for the fungus to mimic termite eggs. In this talk, I would like to demonstrate how the fungus mimics termite eggs so as to control egg protection behavior of the termites. The fungus mimics termite eggs chemically by producing the cellulose-digesting enzyme  $\beta$ -glucosidase (Fig. 2). We found that the termite egg-recognition pheromone consists of  $\beta$ -glucosidase and lysozyme. Both enzymes are major salivary compounds in termites and are also produced in termite eggs. Termite balls were tended by termites only when the fungus produced  $\beta$ -glucosidase. Our results showed that the overlap of the cellulose digestion niche between termites and the fungus sharing the same chemicals provided the opportunity for the origin of termite egg mimicry by the fungus. Our findings suggest that pheromone compounds may have originally evolved within other life history contexts, only later gaining function in chemical communication. Egg mimicry, by which the fungus can easily gain access to the centre of the nest, seems to be an evolutionary loophole around anti-parasite defense in termites.



**Fig. 1** Termite balls in the egg pile of a termite *Reticulitermes flavipes*. Termite eggs are transparent and oval, while termite balls are brown and spherical.



**Fig. 2** Detection of  $\beta$ -glucosidase using a fluorescent probe Micrograph (upper panels) or fluorescent micrographs (lower panels) of a termite salivary gland, termite eggs dissected from the ovary of a queen, cross-section of the abdomen of a termite worker and termite balls harvested from a Potato Dextrose Agar (PDA) plate containing termite nest material. SG: salivary gland, SR: salivary reservoir, SD: salivary duct, HG: hind gut. Termite eggs and termite balls share  $\beta$ -glucosidase, while no  $\beta$ -glucosidase was present in the Argentine ant egg (in the square).

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# Evolution of ancient agriculture in ants

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Host-microbe symbioses have played a critical role in the evolution of biological diversity and complexity. A paradigmatic example is the fungus-growing ant-microbe symbiosis, where coevolution between ants and their microbial symbionts has culminated into one of the dominant herbivores of the Neotropics: leaf-cutter ants (Figure 1). These ants carefully tend their fungal mutualist, providing optimal conditions for growth; in exchange, the fungus serves as the main food source for the colony. The origin of this mutualism occurred more than 45 million years ago, and the subsequent shared evolutionary history has generated significant diversity in both the ants and their fungi.



**Fig. 1.** Leaf-cutter ants use fresh leaf substrate to cultivate their mutualistic fungi. These ants form conspicuous trails of ants carrying leaf fragments within neotropical ecosystems. (photo courtesy of Alex Wild).

Recent work has shown that the gardens of fungus-growing ants are host to a specialized, virulent, and coevolved fungal pathogens called *Escovopsis*. *Escovopsis* is a microfungal genus allied with the order Hypocreales, which derives nutrients from the ants' cultivated fungus. *Escovopsis* is known only from ant fungus gardens and occurs throughout the diversity of the association, indicating that it is obligately associated with the mutualism. The parasite has been found to infect >75% of colonies in natural populations, and has the ability to completely devastate an ant colony, rapidly overgrowing whole gardens. At sub-lethal levels, *Escovopsis* causes a substantial reduction in garden growth and in the production of new workers.

Molecular phylogenetic analyses indicate that *Escovopsis* had a single and early evolutionary origin in the symbiosis, with clades of *Escovopsis* broadly specialized on specific lineages of fungus-growing ants and their fungal cultivar.

To help deal with this garden pathogen, the ants have formed a mutualistic association with Actinobacteria, which produce antibiotics that suppress the growth of *Escovopsis* (Fig. 2). Actinobacteria are Gram-positive, filamentous bacteria well known for their ability to produce potent antibiotics. Indeed, the majority of antibiotics used pharmaceutically are derived from Actinobacteria. Many fungus-growing ants have a conspicuous white deposit on their bodies that traditionally was thought to be a cuticular wax, but in fact is a thick growth of the filamentous Actinobacteria (Fig. 2a-c). Like the cultivated fungi, the bacteria appear to be associated with all species and every nest of fungus-growing ants and are vertically transmitted from parent to offspring nest by queens during nuptial flights. The filamentous bacteria are in the genus *Pseudonocardia*. Bioassays have revealed that the bacteria produce antibiotics that suppress the growth of the specialized parasite *Escovopsis*, but appear to have significantly less effect on general fungi. Experimental work involving the removal of the bacteria from ant workers with antibiotics resulted in fast deterioration of the garden in those nests infected with *Escovopsis*. To protect and nourish the bacteria, the ants have evolved elaborate modifications, including crypts and glands (Fig. 2c-e). Individual ant nests are associated with a single strain of *Pseudonocardia*, but different sympatric colonies from individual species of ants can maintain genetically distinct strains of bacteria. I will present

evidence of an ancient association between the ants and their actinobacterial symbionts and explore the evolutionary dynamics between *Escovopsis* and the tripartite mutualism of ant, fungus and bacteria.

**Fig. 2.** The ant *Cyphomyrmex costatus* showing the specialized location (propleura) of concentrated biomass of white bacterium, just below the head (a&b). SEM of the propleura in *C. muelleri* with bacteria (Fb) (c) and without (d), revealing the crypts for housing the mutualist. (e) Light micrograph of a semi-thin section through the thorax of *C. longiscapus* illustrating the presence of glands (Gl), duct cells (black arrow) associated with the crypts, and concentrations of bacterium (Fb) (Cu, cuticle, bars 50  $\mu$ m; adapted from Currie et al. 2006).

