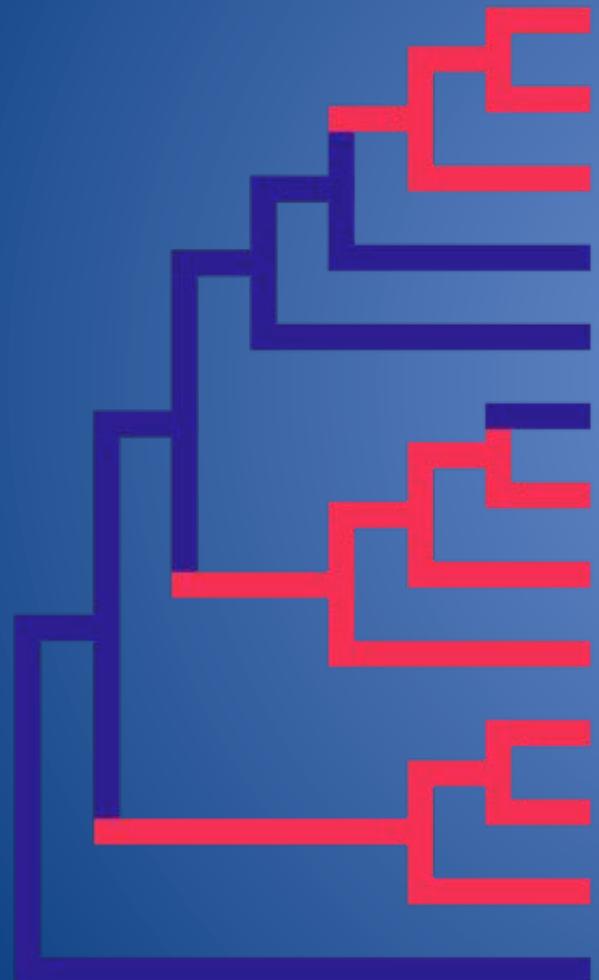


# 種の系統樹と遺伝子の系統樹、 そして表現形質から適応進化を考える： チャルメルソウ類をモデルとして



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奥山 雄大

# 系統樹の真価

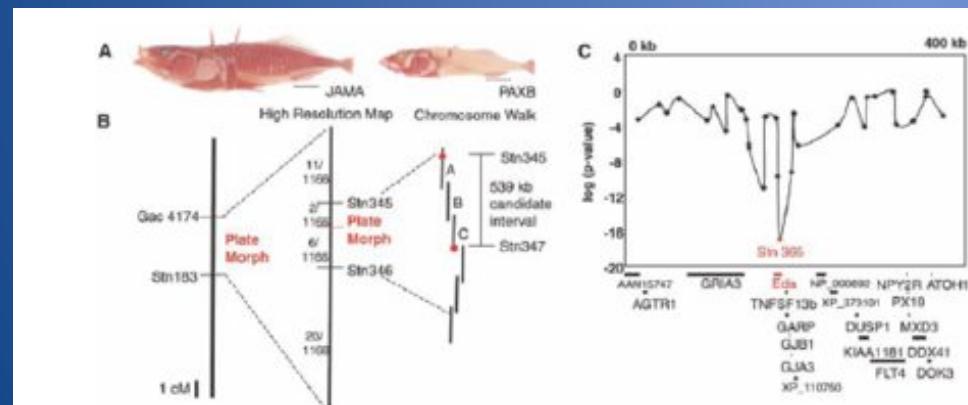


- ・進化イベントの反復が取れる
- ・平行進化を引き起こした共通の生態学的要因が探れる

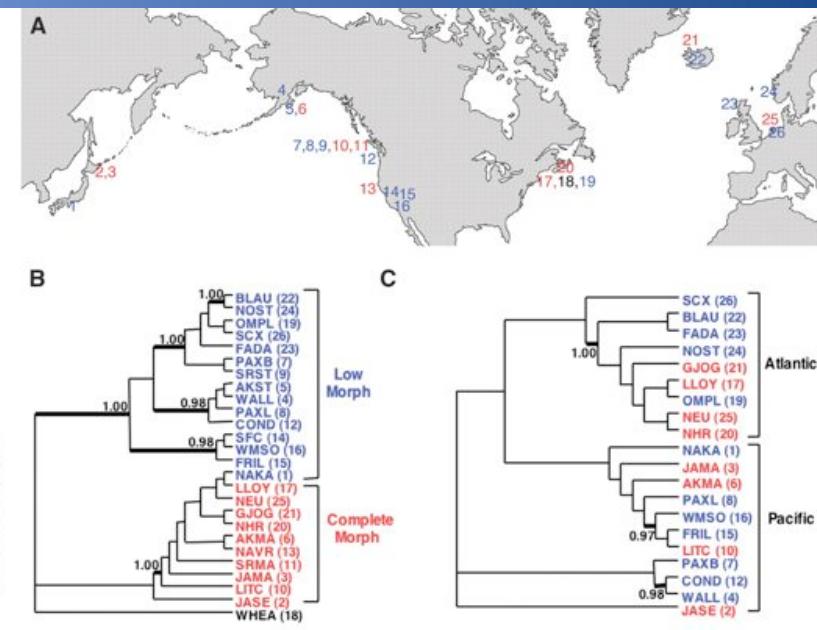
種分化の歴史を  
詳細に反映していることが前提

# 「種の系統樹」(=バックグラウンド)と適応遺伝子の系統樹の不一致

- 新規突然変異の他に遺伝子の水平移動、遺伝子浸透や祖先多型の維持等の適応遺伝子獲得メカニズムが存在
- 種間で異なる選択圧にさらされている遺伝子では種の系統関係から乖離しうる



(C) Linkage disequilibrium screening. Two sequenced BAC clones were used to develop new microsatellite markers (Stn346–Stn379) located 15, 39, 48, 63, 96, 103, 118, 120, 135, 137, 142, 145, 145, 163, 165, 177, 179, 182, 195, 198, 203, 250, 267, 282, 284, 293, 315, 319, 339, 353, 354, and 374 kilobases from Stn345 (black dots). Stn365, located in the stickleback *Eda* locus, showed large differences in allele frequency in completely and low-plated fish from Friant, CA. Positions of other genes in the sequenced interval are shown, with human genome nomenclature committee (HGNC) designations, or accession numbers of the best matches in National Center for Biotechnology Information (NCBI) BLAST searches.



Colosimo *et al.* 2005 Science

適応遺伝子の系統樹

種の系統樹

## チャルメルソウ類(*Heuchera* group)とは

- チャルメルソウ属を含む9属からなるユキノシタ科多年草の单系統群
- 北米の*Heuchera*属(約50種)と日本(および台湾)のチャルメルソウ属チャルメルソウ節(13種)が最も多様化した系統
- チャルメルソウ節は日本の渓流環境に適応した系統



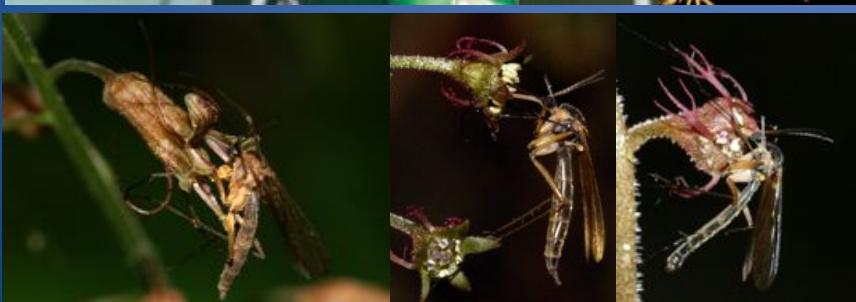
# チャルメルソウ類における 送粉様式の多様性



ハナバチ・ハナアブ・ガ媒  
林床/草地/岩場



キノコバエ媒  
渓流沿い/林床



シギキノコバエ媒  
渓流沿い

多湿な環境

# チャルメルソウ類の送粉者となる キノコバエ類には2タイプある

**筒状一釣鐘状の花**

ミカワチャルメルソウ  
チャルメルソウ  
タキミチャルメルソウ  
シコクチャルメルソウ  
*Tolmiea menziesii* (US)

**皿状一杯状の花**

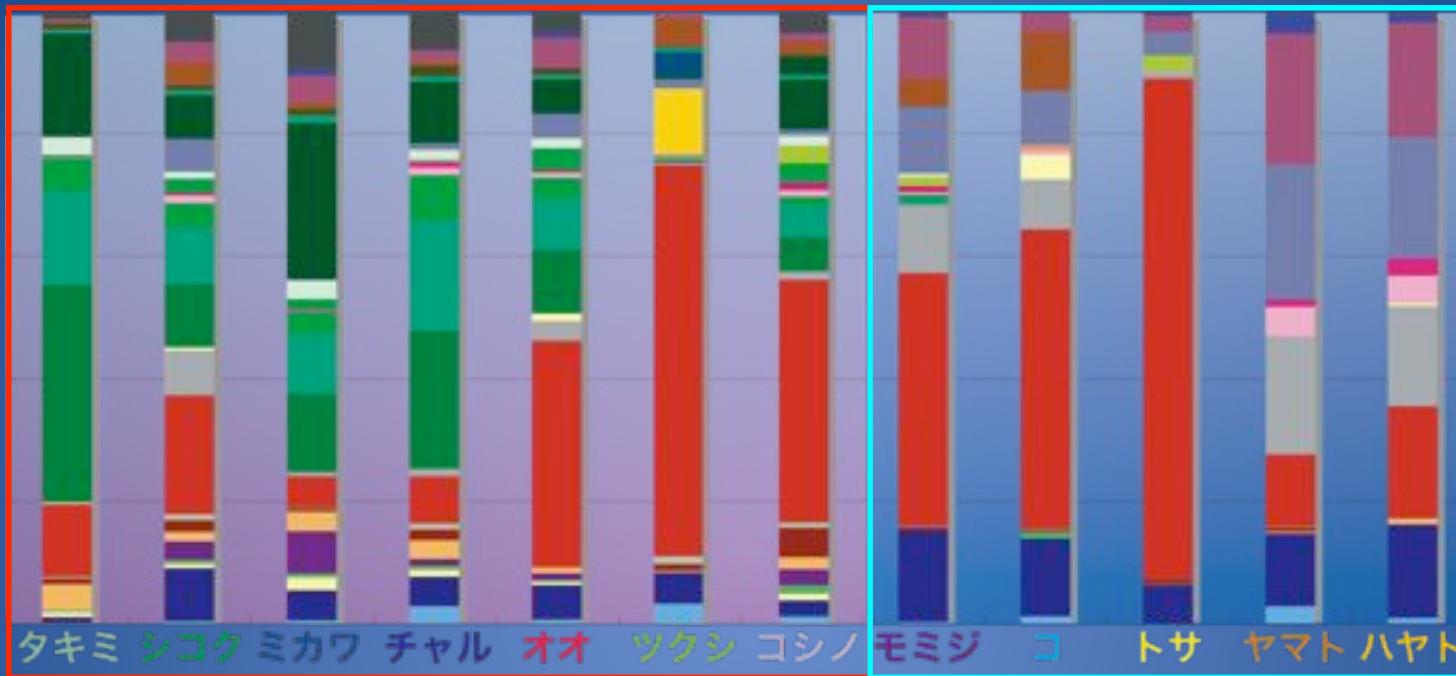
モミジチャルメルソウ  
オオチャルメルソウ  
ツクシチャルメルソウ  
コシノチャルメルソウ  
ハヤトチャルメルソウ (新種)  
*Mitella pentandra* (US)  
*Mitella breweri* (US)  
*Mitella caulescens* (US)  
コチャルメルソウ  
ヤマトチャルメルソウ (新種)  
マルバチャルメルソウ  
エゾノチャルメルソウ

**シギキノコバエ**

**口吻が伸長していない  
キノコバエ**

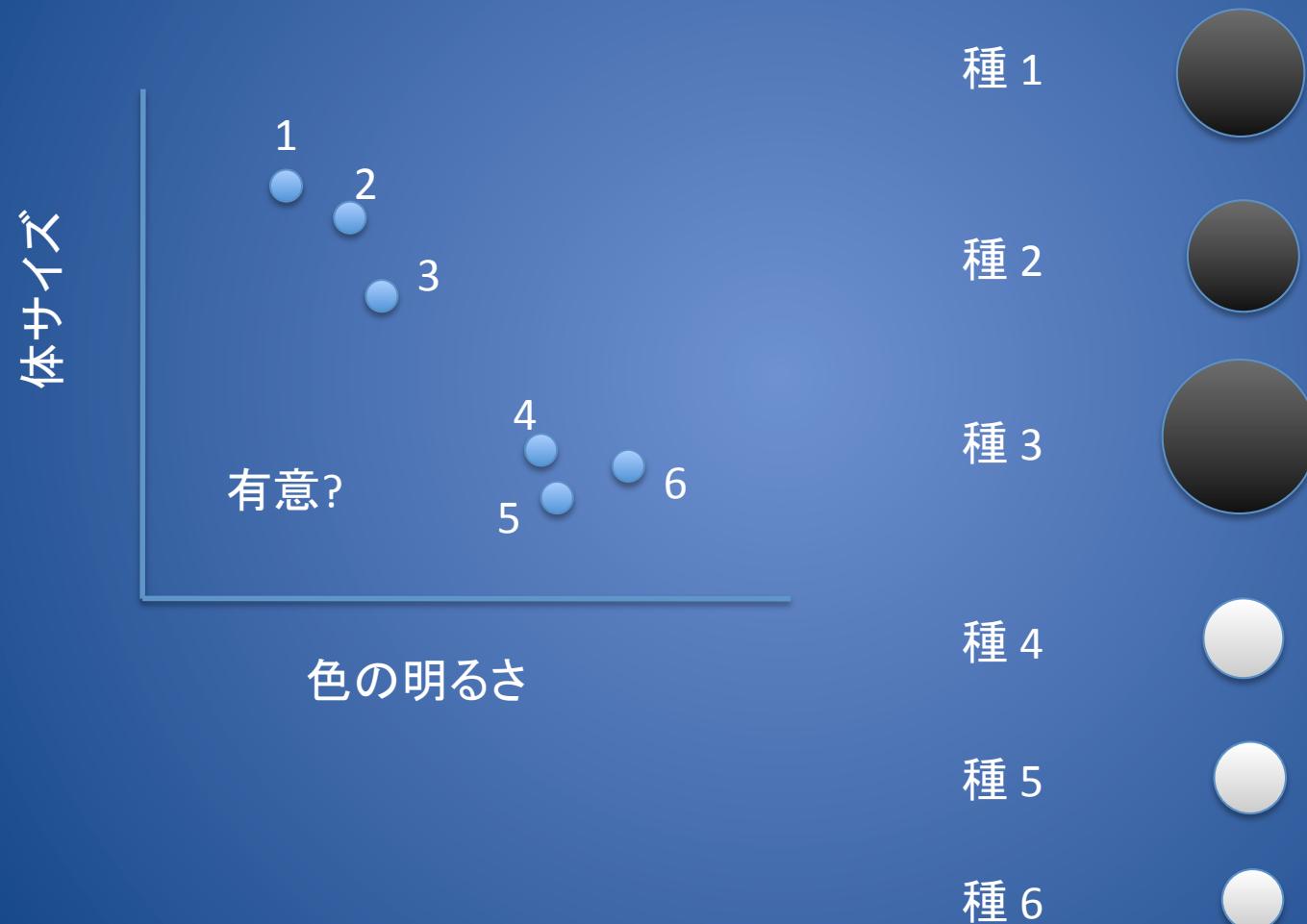
# チャルメルソウ全種の花香成分組成

シギキノコバエがよく訪花する種



シギキノコバエがあまり訪花しない種

# 種間で形質間の相関を調べる際に生じる問題

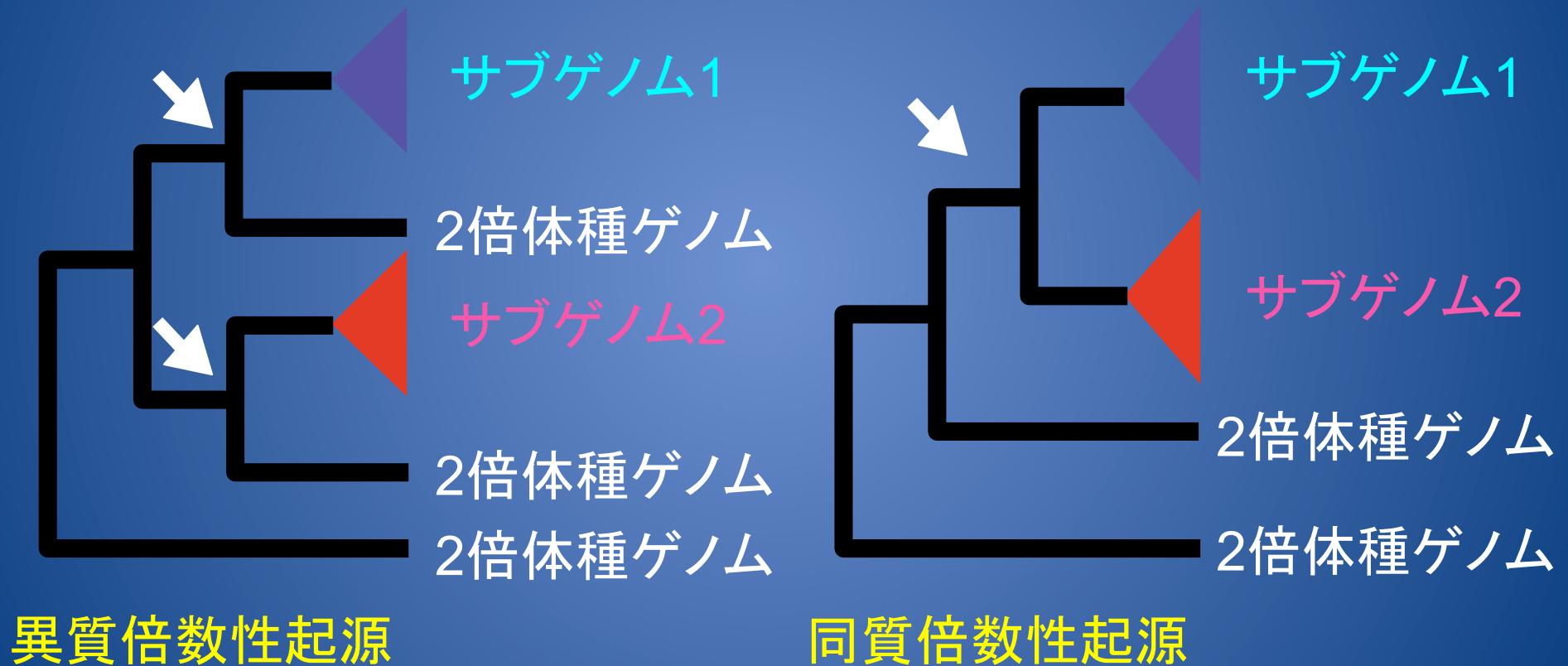


# チャルメルソウ節の起源と種の系統関係を 解明

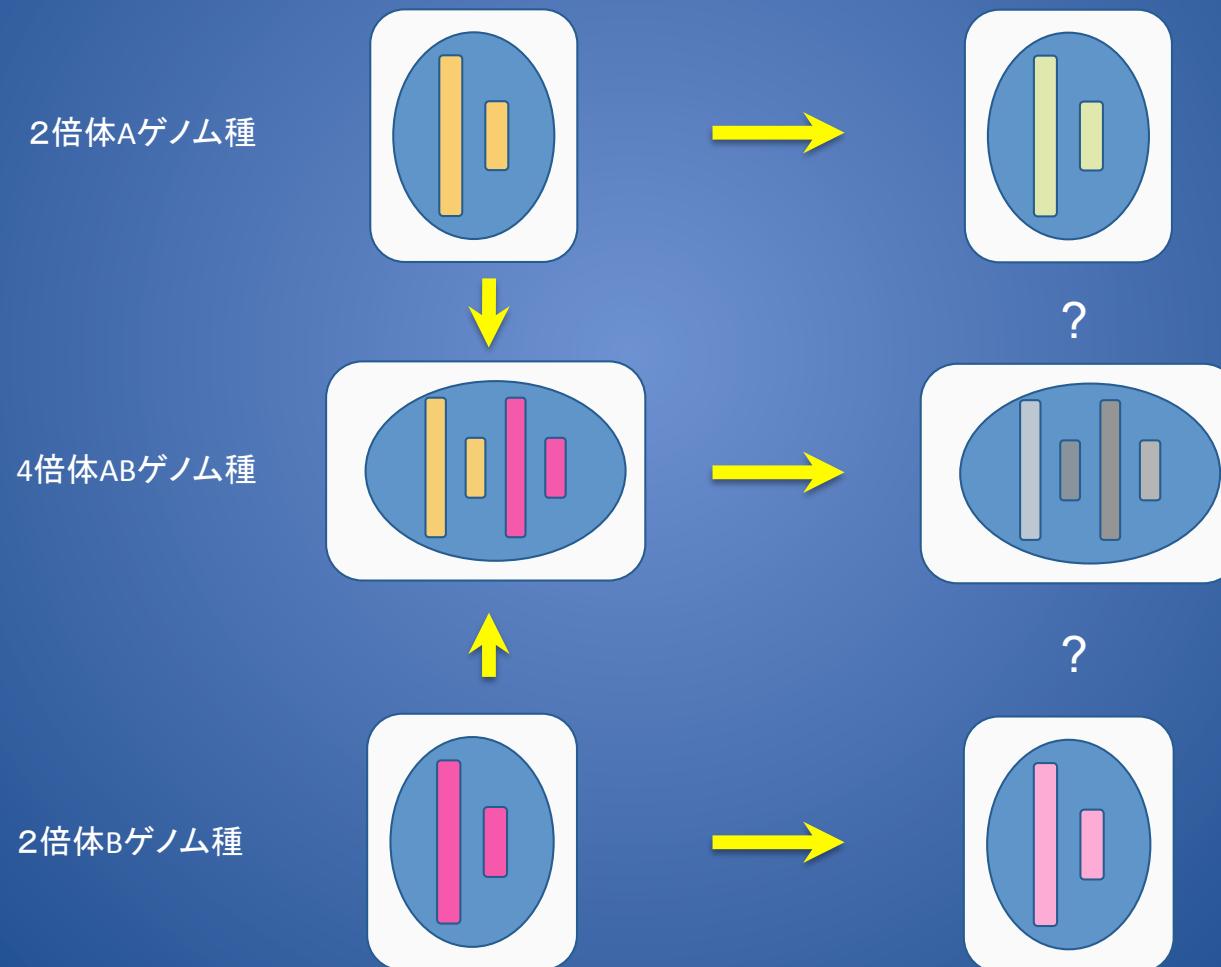
13/39



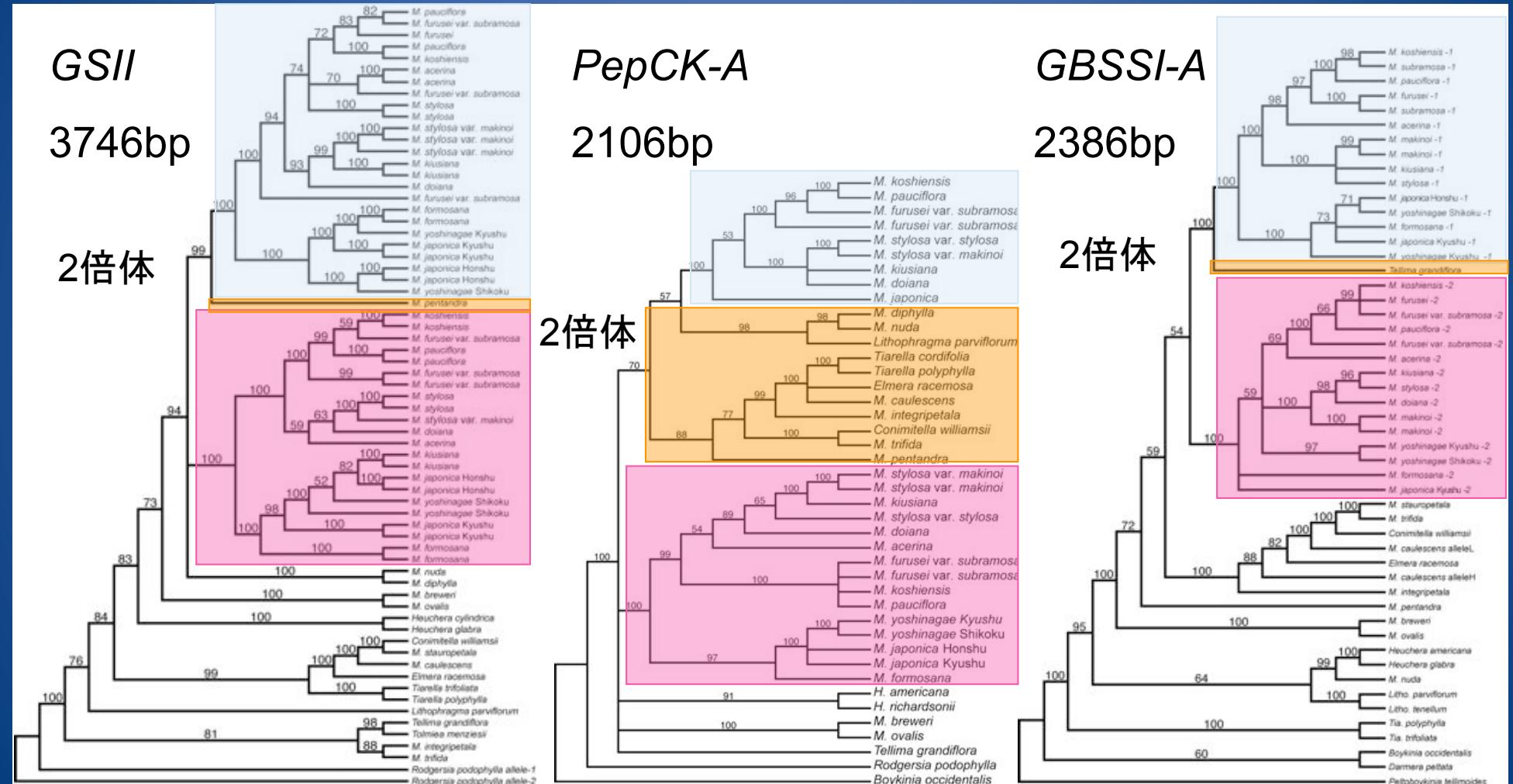
# 重複遺伝子の系統樹を用いた 倍数化ゲノムの起源の解析



# 一般に古い倍数化イベントの復元は困難である



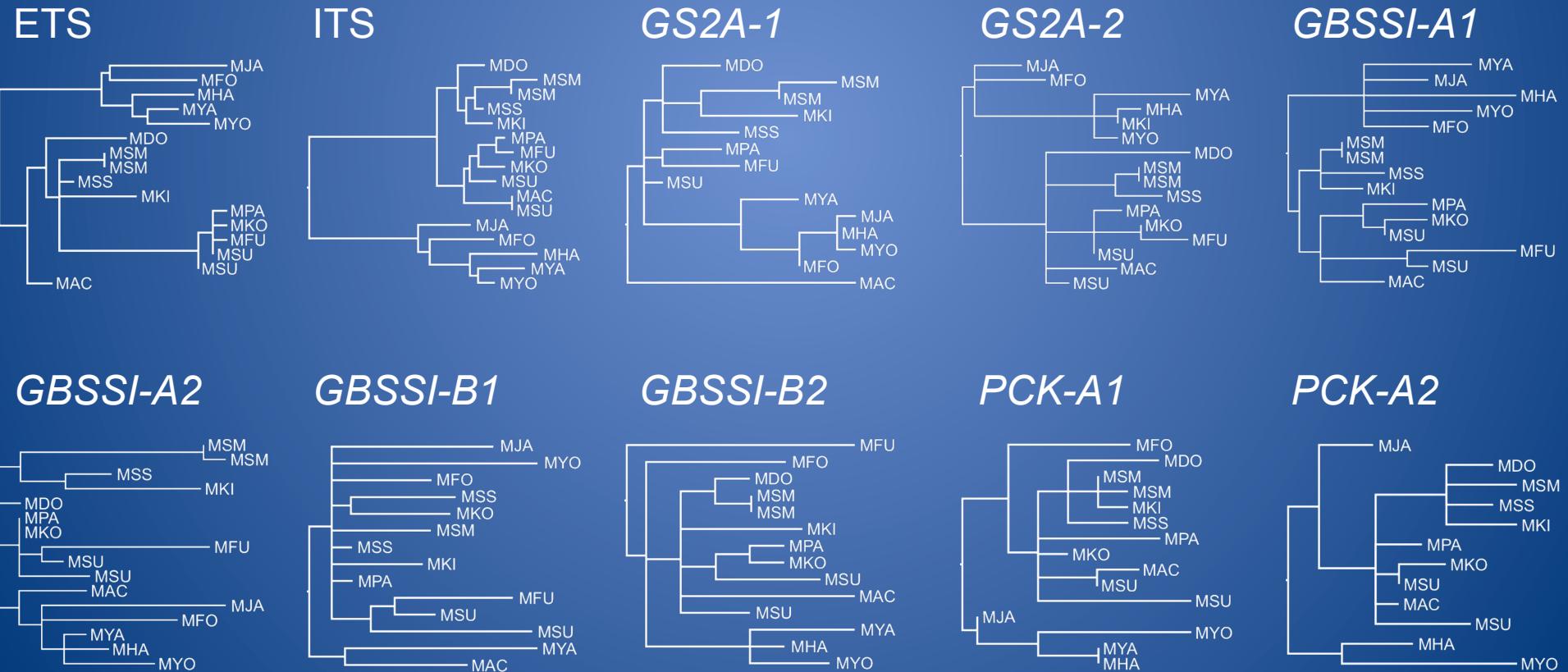
# チャルメルソウ節は、 ただ一回の異質倍数化から多様化した



だが、遺伝子ごとに2倍体祖先種との系統関係が異なる、、、真相は？

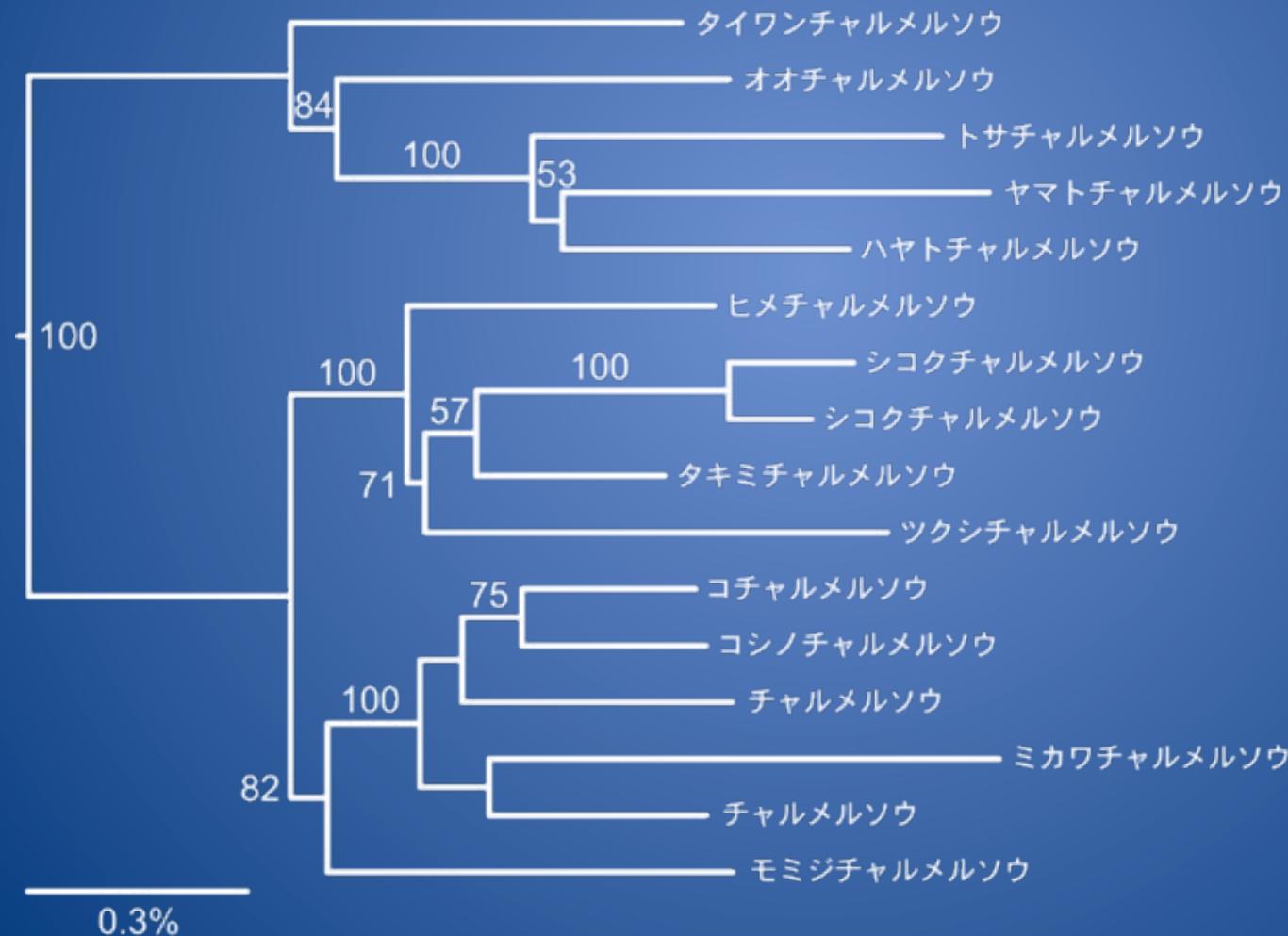
# チャルメルソウ節全種の 超高精度分子系統樹の構築

- ・ バックグラウンドの進化史を詳細に明らかにする
- ・ 相同な核10遺伝子座計8kb以上をほぼ全種で単離



# チャルメルソウ節全種の 超高精度分子系統樹の構築

- 核10遺伝子座8kbのデータを結合、最尤法により系統樹を構築

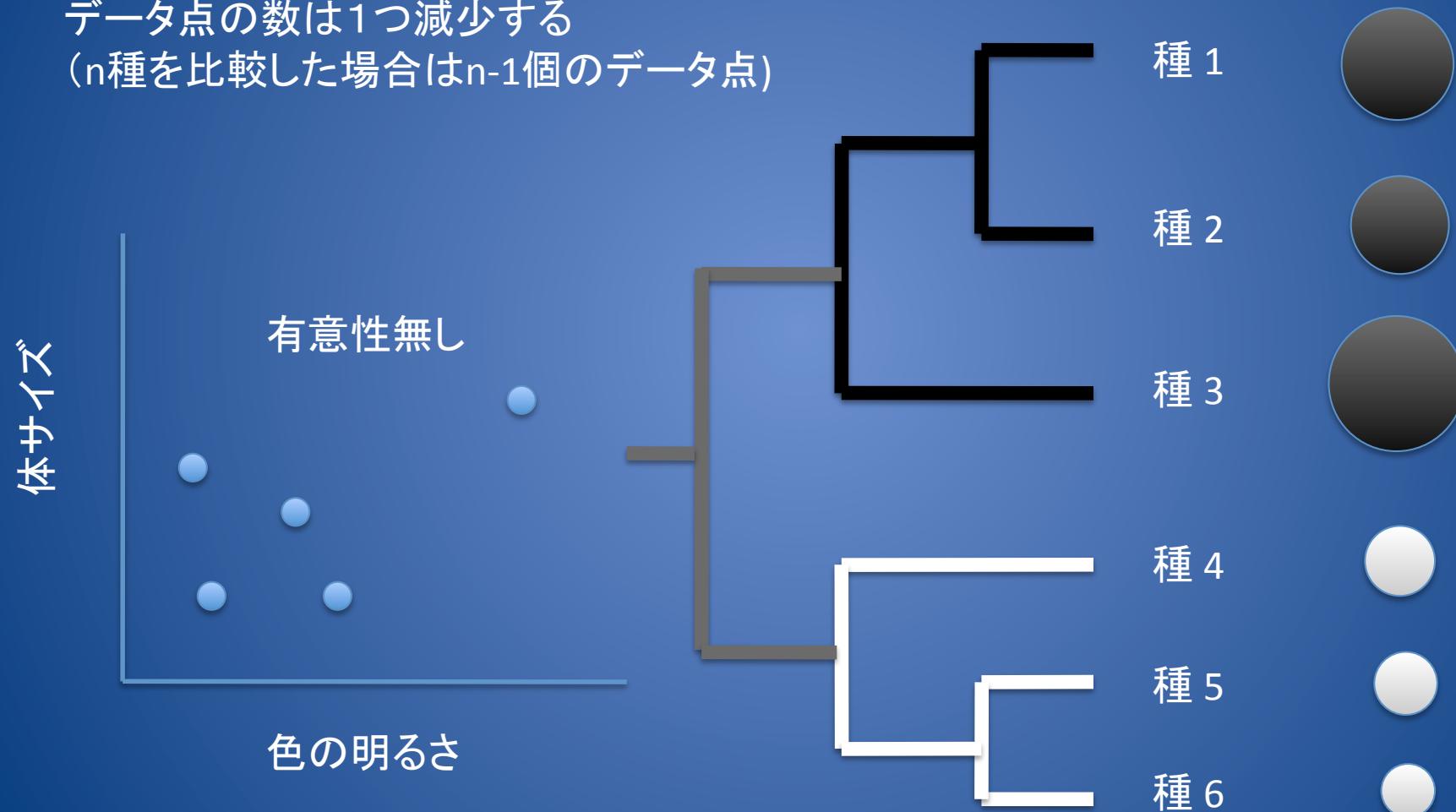


# 系統学的独立比較: PIC

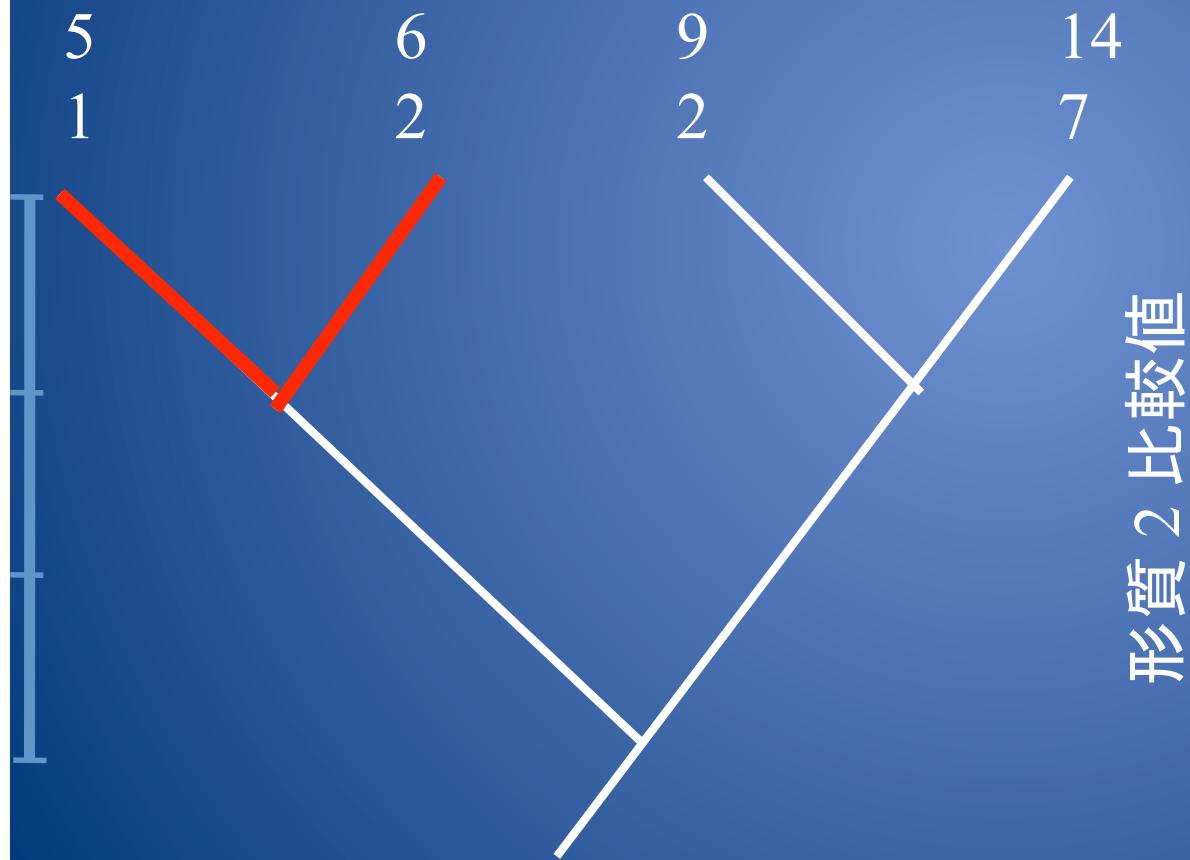
データ点の値から系統関係の影響を差し引く

データ点の数は1つ減少する

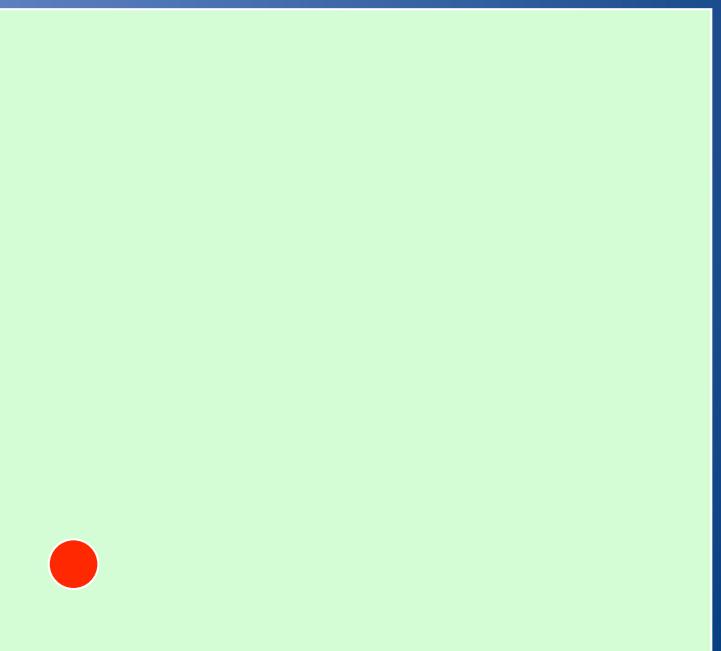
( $n$ 種を比較した場合は $n-1$ 個のデータ点)



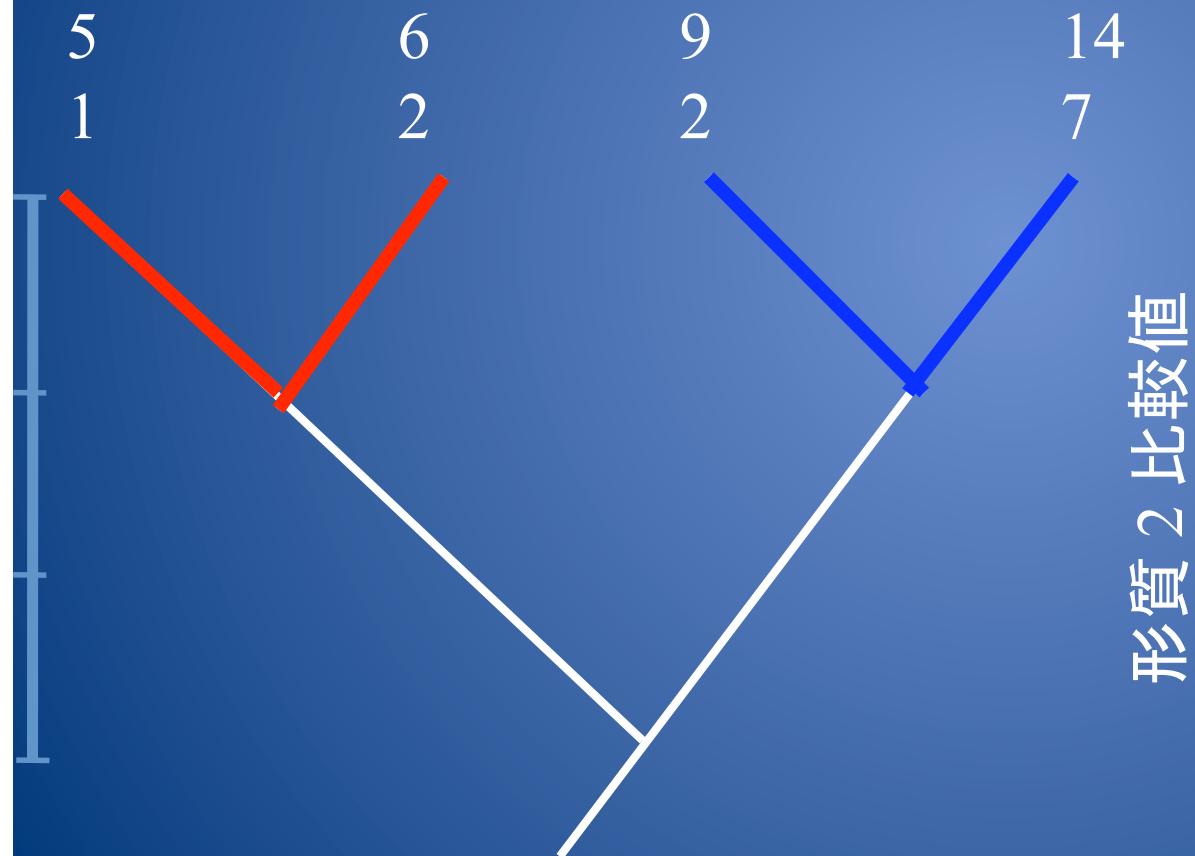
# 系統的独立比較の実際



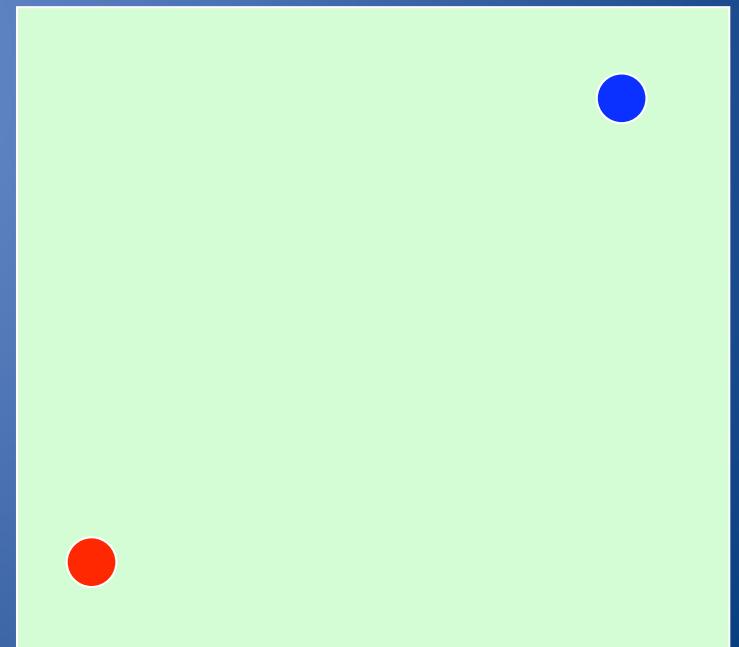
形質 1:  $(6-5=1)$   
形質 2:  $(2-1=1)$   
比較値:  $(1,1)/\sqrt{2}$



# 系統的独立比較の実際

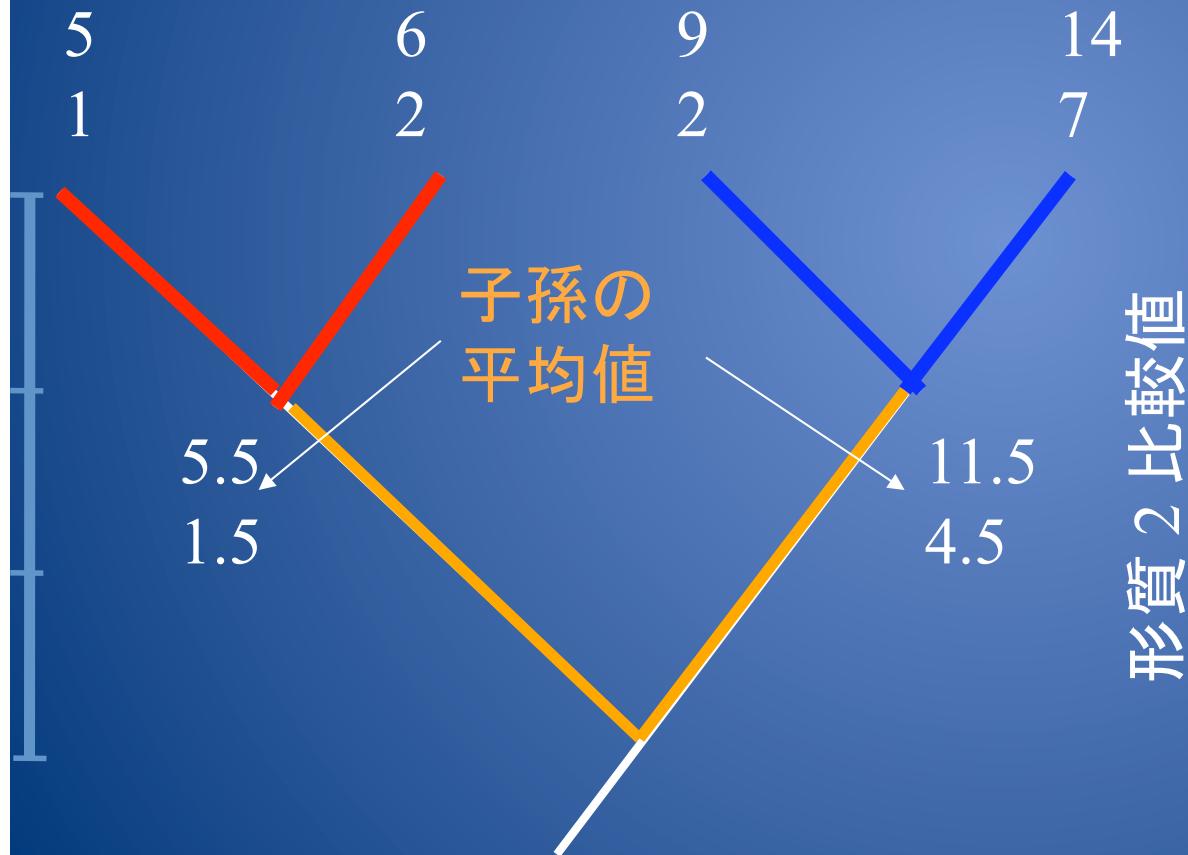


形質 1:  $(14-9=5)$   
形質 2:  $(7-2=5)$   
比較値:  $(5,5)/\sqrt{2}$

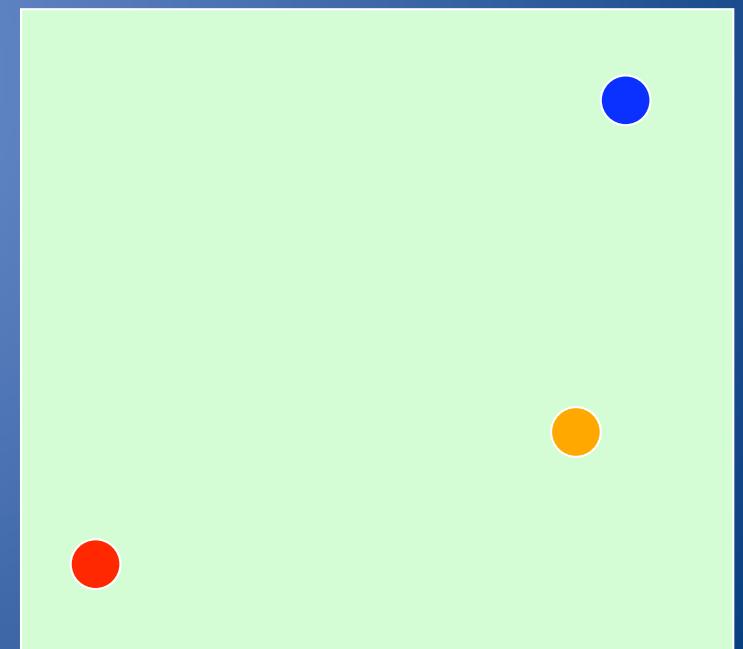


形質 1 比較値

# 系統的独立比較の実際



形質 1:  $11.5 - 5.5 = 6$   
形質 2:  $4.5 - 1.5 = 3$   
比較値:  $(6, 3) / \sqrt{4}$



形質 1 比較値

# やってみよう系統的独立比較

## 必要なもの

- なるべくすごい系統樹(PAUP\*などのソフトウェア)
- 2種類以上の量的形質データ
- Mesquite
- PDAP/PDTREE パッケージ

The image shows two screenshots of software pages. The top screenshot is the Mesquite homepage, featuring a logo of a green plant, the text 'MESQUITE A modular system for evolutionary analysis', and a brief description of its features. The bottom screenshot is the PDAP:PDTREE application page, which includes a logo, a brief description, and a link to the documentation.

**MESQUITE** A modular system for evolutionary analysis  
M.P. Maddison University of British Columbia  
D.A. Maddison University of Arizona

If you're looking for Mesquite Software, Inc., or its CSIM 18 toolkit for building simulation models, go [here](#).

Current version: 2.6

Mesquite is software for evolutionary biology, designed to help biologists analyse comparative data about organisms. Its emphasis is on phylogenetic analysis, but some of its modules concern population genetics, while others do non-phylogenetic multivariate analysis. Because it is modular, the analyses available depend on the modules installed. Analyses include:

- Reconstruction of ancestral states (maximum likelihood)
- Tests of processes of character evolution, including correlation
- Analyses of speciation and extinction rates
- Analysis of phylogenetic relationships (maximum likelihood, DNA, continuous)
- Parametric bootstrapping (integration with programs such as RAxML™ and NONA)
- Morphometrics (PCA, CA, geometric morphometrics)
- Tree comparisons (e.g. tree distance, Ancestral state prediction)
- Tree comparisons and simulations (tree similarity, Ancestral state prediction)

There is a brief [outline of features](#), which includes screenshots. Mesquite is not primarily designed to infer phylogenetic trees, but rather for diverse analyses using already inferred trees.

The Mesquite [manual](#) describes in more detail [what Mesquite can do](#). The manual is in the download, or can be browsed [here](#), and is also available as a [printable pdf](#) (up to date as of as of version 2.6).

**PDAP:PDTREE: A translation of the PDTREE application of Garland et al.'s Phenotypic Diversity Analysis Program**

Peter E. Midford, Theodore Garland Jr., and Wayne P. Maddison

First released September 2003  
Current release November 2008

**Important Note**

Recent versions of PDAP:PDTREE (1.05-1.14) will calculate statistics for characters that have missing values. When there are no missing values, all statistics have been tested and are, to the best of our knowledge, correct. When there are missing values, certain statistics listed at the bottom of the test pane of Screeners 9-11 have been tested and are believed to be correct. Likewise, the first 5 columns of PDC files have been tested and are believed to be correct.

Unfortunately, certain values, such as later PDC file columns and the contrast values that appear in the scatterplots of screeners 9-11 are not known to be correct. We are continuing to work on correcting these issues and hope to have a complete solution soon. If these values are of concern to you, you can always prune your tree down to the number of tips for which you have complete data for two given traits, and save the file. Analyses with contrasts should then be correct for those two traits.

**Documentation**  
Documentation in MS word format available [here](#).  
Documentation in PDF format available [here](#).

**News**

25 November 2008 - Version 1.14 released. This version introduces a flag to ignore intensities in the nexus, which are produced by some tree inference programs, updates the guided tour and other examples, adds character names to diagnostic tests for writing distance matrices. Thanks to Wayne Maddison suggesting and overseeing to these enhancements. This release also incorporates a bug reported by David Maddison when loading a nexus file that had been saved with screen 9 deleted.  
10 July 2008 Version 1.12 released. This version supports Mesquite 2.5. Most screens support the "Skip Through Trees..." command. Any remaining chart scaling issues with screens 9A and 9B (the root reconstruction and regression screens) should now be resolved. The new version of the program is compatible with the PDAP support for the phenomic version checking; we are working on a solution for these.  
16 December 2007 Version 1.1 released as bug fix and minor enhancement.  
21 September 2007 Version 1.09 released for Mesquite 2 compatibility and as bug fix.  
19 September 2007 Version 1.08 released as bug fix.  
17 December 2006 - Version 1.08 released as bug fix.  
15 December 2006 - Version 1.08 released as bug fix and up released. Numerous bug fixes and the ability to change the degrees of freedom on screen 9 have been added. The latter allows calculation of a polymomy corrected p-value for the regression test.  
19 October 2005 - Version 1.07 released as bug fix.  
26 August 2005 - Version 1.07 for Mesquite 1.04 and up released. C1 files and several new scripting commands added along with two important bug fixes for exported files.  
8 March 2005 - Version 1.06 for Mesquite 1.04 and up released. Bug fixes and enhancement to PDF file export.  
13 October 2004 - Version 1.05 released as bug fix.  
23 May 2003 - Update for 0.995 released. Fixed error in sign test calculation.  
18 February 2003 - Provide a way to avoid rendering user info on this page.  
10 February 2003 - Provide a way to avoid rendering user info on this page.  
26 November 2002 - Source (.java) is now available on the download page.  
15 November 2002 - Update with several bug fixes.

# 系統樹を読み込みMesquiteでデータを入力

DATA  
SPLIT TREES:  
Title "Trees from "Rishitoku01.tre"  
LINK Taxon = Taxon;  
TRANSLATE  
1 M\_YAMATO\_R2,  
2 M\_japonica\_T,  
3 M\_HAYATO\_I,  
4 M\_yoshinagae\_KW2,  
5 M\_formosana\_R2,  
6 M\_doliana\_Y5,  
7 M\_stylosa\_var\_makinoi\_001,  
8 M\_stylosa\_var\_stylosa\_K21,  
9 M\_kiusiana\_I,  
10 M\_pauciflora\_A,  
11 M\_koshiensis\_M,  
12 M\_furusei\_var\_furusei\_H3,  
13 M\_acerina\_AS,  
14 M\_furusei\_var\_subrami\_AS1;  
TREE PRUP.t = ((10,0.005092,(12+0.005318,0+0.004927,((6+0.004266,(C7+0.005129,8+0.002498)7.71E-4,9+0.006395)z2,0.94E-4)10-  
END;

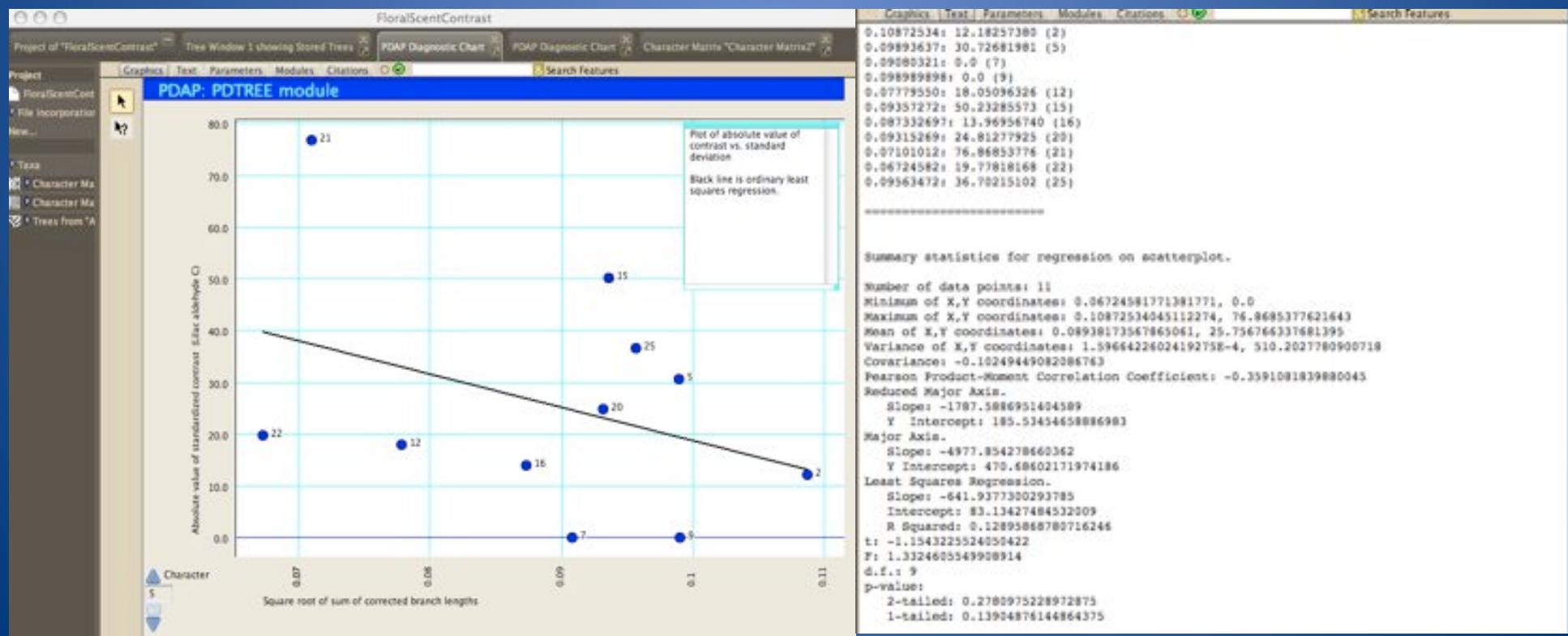
The screenshot shows the Mesquite software interface with the following details:

- Title Bar:** フォーマティティ (Format) - FloralScentContrast
- Project:** Project of "FloralScentContrast"
- Tree Window:** Tree Window 1 showing Scaled Trees
- Diagnostic Charts:** PDAP Diagnostic Chart 1, PDAP Diagnostic Chart 2
- Character Matrix:** Character Matrix "Character Matrix"
- Table Content:** A character matrix table titled "Taxon 1: Character". The table has 14 rows (taxa) and 29 columns (characters). The columns are numbered 13 through 29. The first few rows of data are as follows:
 

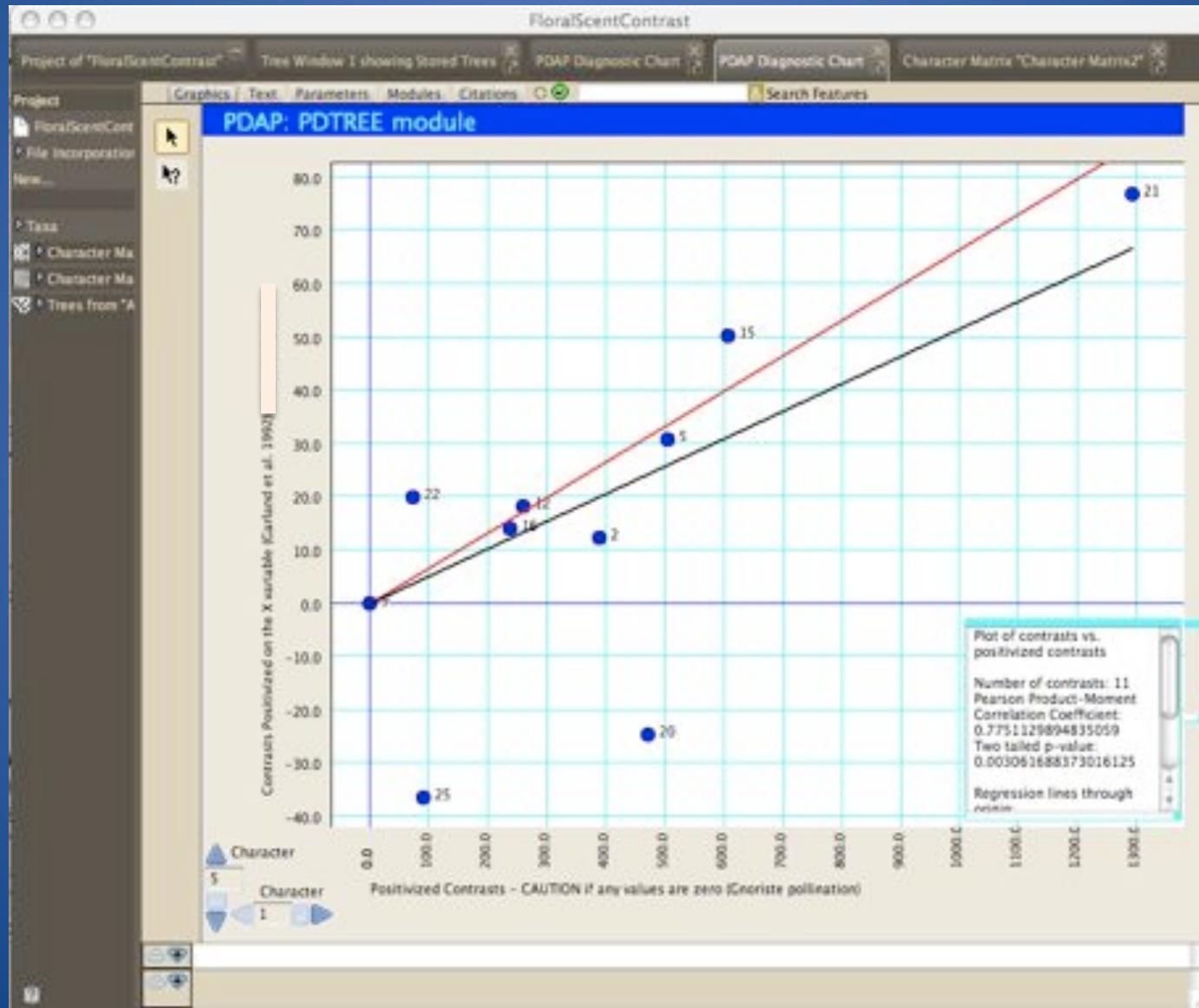
	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29		
1 M_YAMATO_R2	0.19	5.22	1.21	19.14	0.0	23.43	0.0	0.0	0.0	0.0	54.65	42.57	0.0	22.08	3.89	0.0	0.0	0.0	
2 M_japonica_T	0.37	0.84	0.15	2.45	0.0	5.5	1.91	0.0	1.44	5.28	0.78	14.26	7.95	0.02	5.37	1.32	3.1	1.1	0.28
3 M_HAYATO_I	4.83	4.64	2.66	16.3	0.0	19.88	0.0	0.0	0.0	0.0	51.48	36.18	0.03	18.73	1.5	0.26	0.43	0.0	0.0
4 M_yoshinagae_KW2	0.19	0.13	0.02	1.2	2.48	3.83	0.0	0.0	0.0	0.0	11.41	5.03	0.0	2.62	0.62	0.02	0.0	0.05	0.0

# 系統的独立比較の仮定と適合するかを確認 (PDAP diagnostic chart)

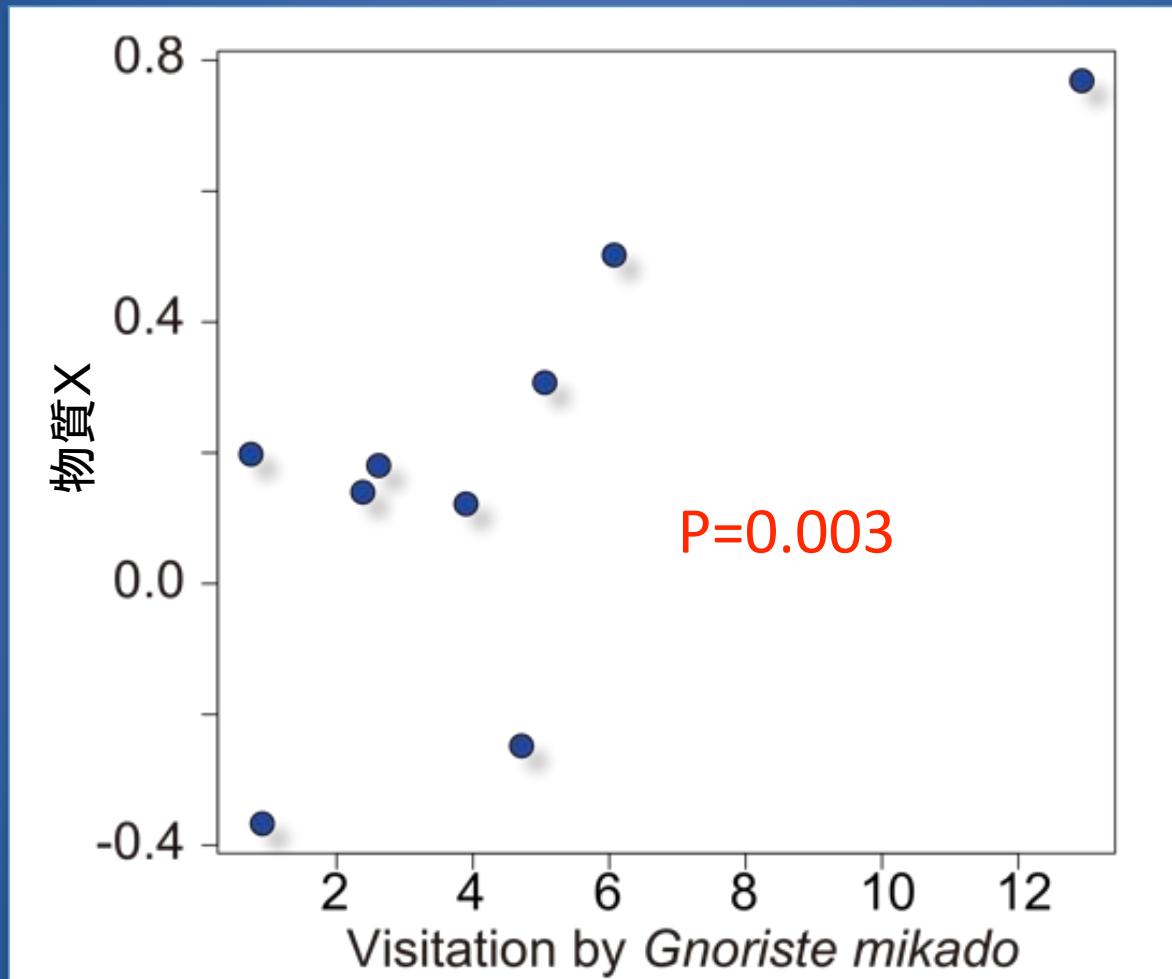
- 標準化した比較値と樹長が相関しない
- 比較値(Y軸の値)は正規分布している
- 明らかな外れ値が存在しない



# X軸とY軸を比較 (PDAP Chart > Y Contrasts vs X Contrasts)



# 系統的独立比較による花の匂いの適応の検出



物質Xがシギキノコバエを誘引している原因物質である可能性は極めて高い

# 遺伝子発現解析法

ノーザンブロット解析法

RT-PCR法

マイクロアレイ法

EST解析

SAGE法

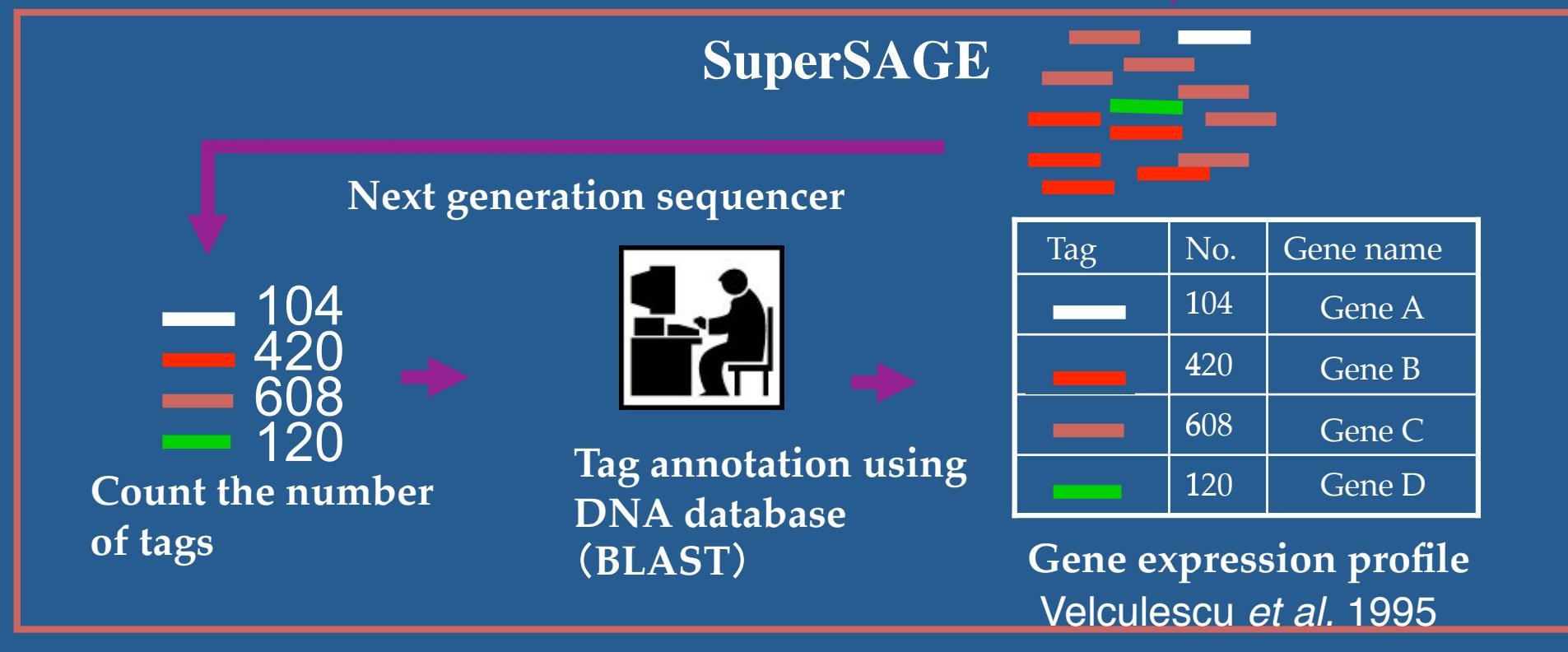
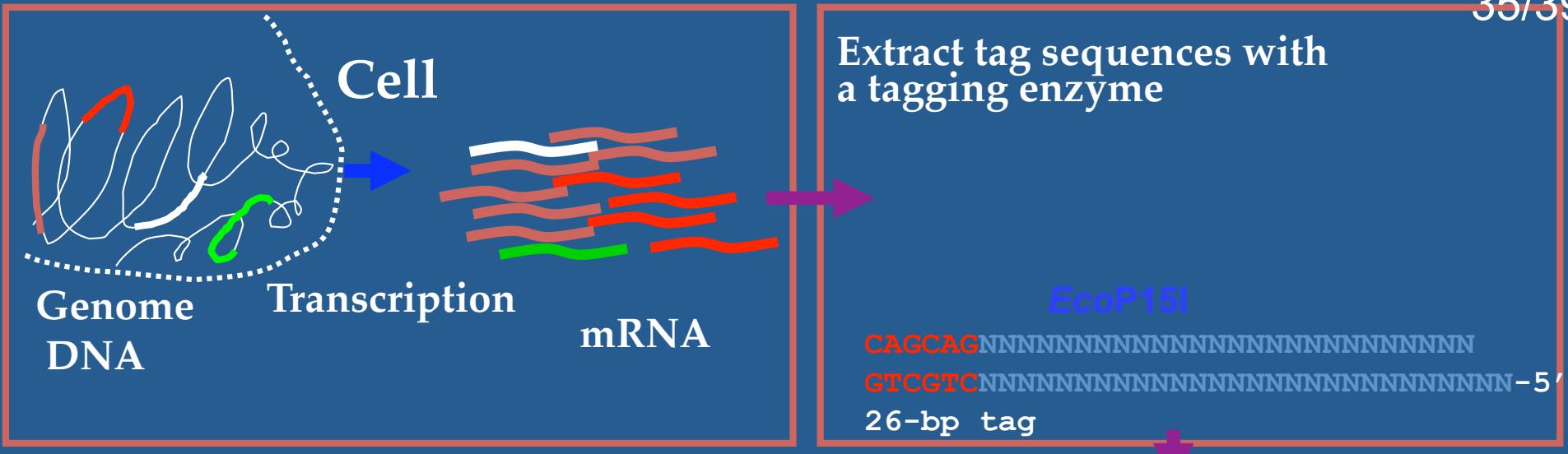
MPSS法



少数個の遺伝子が対象

ハイブリダイゼーション  
に基づく方法

mRNAを数える方法



# SuperSAGEと他の遺伝子発現解析技術の性能比較

	定量性	多検体解析能	ゲノム関連情報の必要度	専用機器
SuperSAGE	高い	かなり高い	不要	不要
SAGE	高い	低い	必要	不要
Microarray	中	高い	必要	必要
MPSS	高い	低い	必要	必要