

Elucidating evolutionary relationships in *Fritillaria* (Liliaceae)

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Introduction

□ The c.140 species of *Fritillaria* are autumn germinating/spring flowering bulbous plants (Figs 1 & 2 show examples); the genus encompasses species of both medicinal and horticultural importance. Members of *Fritillaria* are found in the Northern Hemisphere's temperate zones of North America, Eurasia and North Africa.

□ The most recent classification¹ recognised eight subgenera, with further sections and series within these. This classification was broadly supported by the results of a previous phylogenetic study² based on plastid and nuclear ribosomal region DNA sequence data, which recovered two major clades – one containing Eurasian, North American and North African species (seven subgenera) and the other subgenus containing mainly North American species.

□ The discovery of giant genomes in several *Fritillaria* species^{3,4} has stimulated recent research on acquiring better knowledge of species relationships. Through recovery of increasingly resolved and well supported molecular phylogenetic trees for *Fritillaria*, a greater understanding of the relationships between species and subgenera is being achieved. This is an essential step in providing an evolutionary framework within which to study the mechanisms and evolutionary forces involved in genome size change, as well as forming an important part of wider studies of the genus.

□ Sequence data for 37 *Fritillaria* species (c.26% of the genus) were included in the previously published phylogenetic work². In the current study, to date, DNA sequence data has been obtained for c.160 individuals, representing c.100 species (c.71% of the genus), as well as for 15 sister group (*Lilium*) and two outgroup species. Phylogenetic analysis, using maximum parsimony, has been performed.

□ The latest results of this molecular phylogenetic work aimed at elucidating relationships between all species of *Fritillaria* are presented. Implications of these findings for our understanding of species relationships within the genus are discussed.

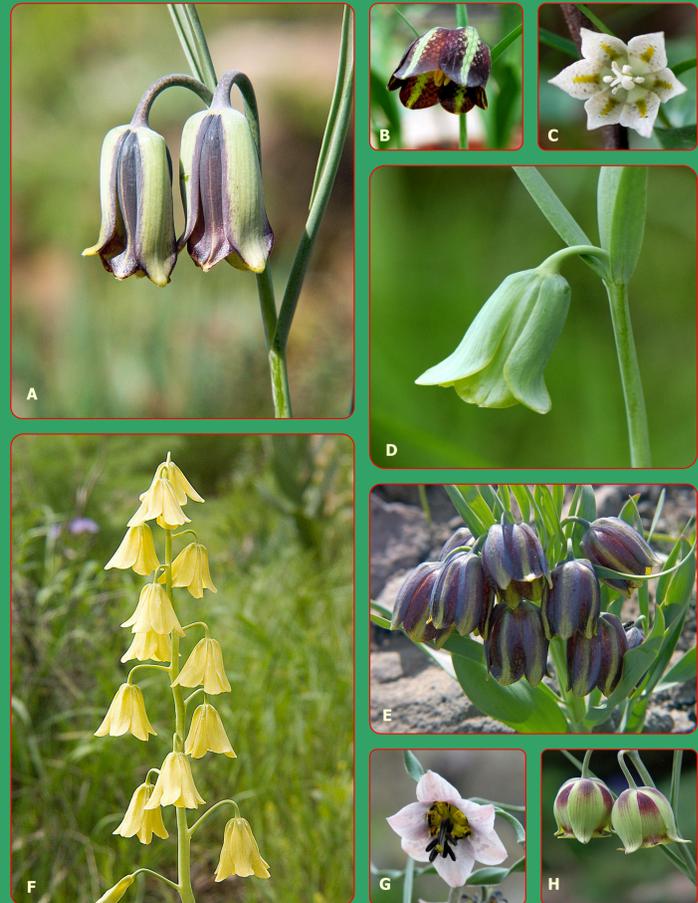


Figure 2. Examples of *Fritillaria* species sequenced: A) *F. latakensis* (subgen. *Fritillaria*); B) *F. thessala* (subgen. *Fritillaria*); C) *F. kalerensis* (subgen. *Japonica*); D) *F. atreidae* (subgen. *Fritillaria*); E) *F. crassifolia* (subgen. *Fritillaria*); F) *F. persica* (subgen. *Theresia*); G) *F. gibbosa* (subgen. *Rhinopetalum*); H) *F. acropetala* (subgen. *Fritillaria*)
Photos A, E, F and H – R. Charman; B, C, D and G – L. Hill

Methods

□ Three regions from the plastid genome (the *rpl16* intron and portions of the *rbcL* and *matK* genes) were amplified and sequenced from all available species and subspecies of *Fritillaria* (some examples sequenced are shown in Figs 1 & 2), including multiple individuals where possible. Representatives of *Lilium*, sister to *Fritillaria*, were also sampled.

□ In addition to the plastid regions, the utility of the nuclear ribosomal ITS region was assessed; with results which showed that for *Fritillaria*, there is evidence for multiple sequence variants of ITS in many individuals, which limits the utility of this region for phylogenetics. For this study, inference of species relationships was therefore based only on the plastid sequence data.

□ Phylogenetic trees inferred from individual plastid regions were compared for congruence before combining the data into a single plastid dataset. Maximum parsimony analysis was carried out using PAUP* v4.0 b10⁵.

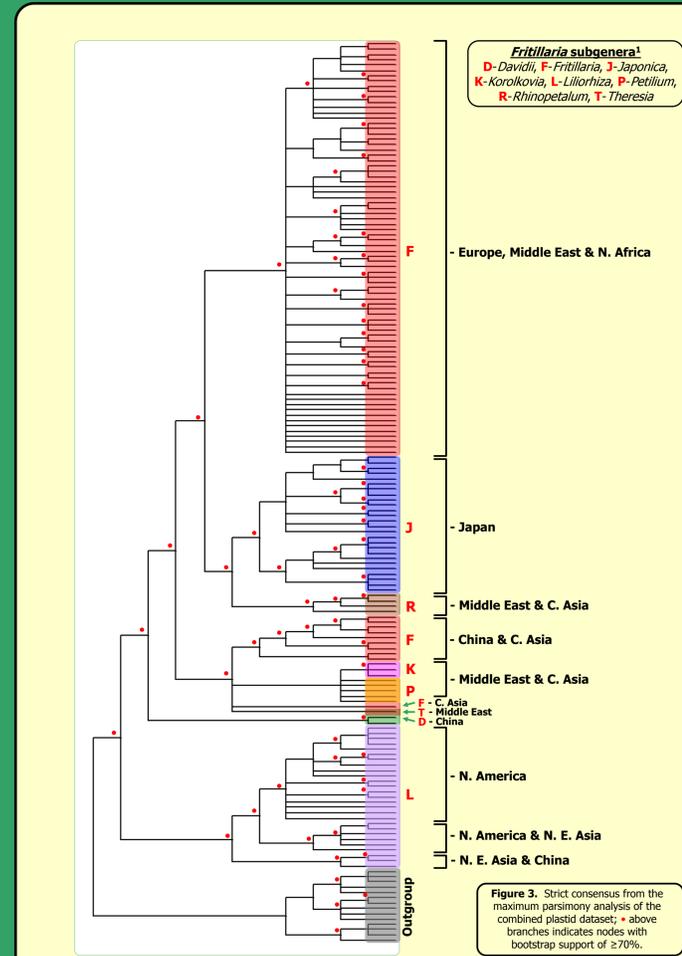


Figure 3. Strict consensus from the maximum parsimony analysis of the combined plastid dataset; * above branches indicates nodes with bootstrap support of $\geq 70\%$.

Results

□ Preliminary analysis of a combined plastid dataset of 101 species from 161 individuals of *Fritillaria* (c.71% of the genus), for only those taxa that had data for all three regions, recovered several well supported clades that correspond to subgenera recognised in the current classification¹ (Fig.3).

□ The largest subgenus, *Fritillaria*, which contains c.70% of the genus (c.100 species), is confirmed to be polyphyletic. The majority of species sampled from this subgenus (56) form a well supported clade, comprising taxa that occur in Europe, the Middle East and North Africa (Fig.3). The remaining eight species sampled from subgenus *Fritillaria*, form a group with the three small subgenera *Korolkovia*, *Petilium* and *Theresia* which occur in the Middle East and Central Asia; seven of these subgen. *Fritillaria* species form a well supported clade of taxa occurring in China and Central Asia (Fig.3). Species with the very largest plant genomes – found within subgen. *Japonica* – form a monophyletic group.

Discussion

□ Based on data from the plastid genome, three of the five subgenera of *Fritillaria* that contain more than one species¹ are shown to be monophyletic – each with good support (Fig.3).

□ Increased sampling of subgen. *Fritillaria* (64 in the current work, compared with 15 previously) has confirmed that the species are split into at least two distinct groups, revealing a small, but well supported clade of Chinese and Central Asian species, that is not closely related to the members of subgen. *Fritillaria* that are found within the large European, Middle Eastern and North African clade.

Future work

□ By studying *Fritillaria* morphological characters, such as flower number, bulb and style type (Fig.5) on the combined plastid tree, ancestral character states will be reconstructed and evolution of continuously varying *Fritillaria* traits will be traced by analysing change, correlation and repeated patterns.

□ Population genetics studies will focus on the origin, routes of migration and genetic diversity of the putative British native *F. meleagris* (snake's head fritillary) (Fig.4) in the UK and Europe. Genetic variation is partitioned amongst populations; and through analysis of plastid DNA microsatellite markers (short tandem repeat polymorphisms), patterns of distribution and divergence will be studied, and implications for species conservation evaluated.



Figure 1. *F. imperialis* (subgen. *Petilium*) R. Charman



Figure 4. *F. meleagris* (subgen. *Fritillaria*) L. Hill

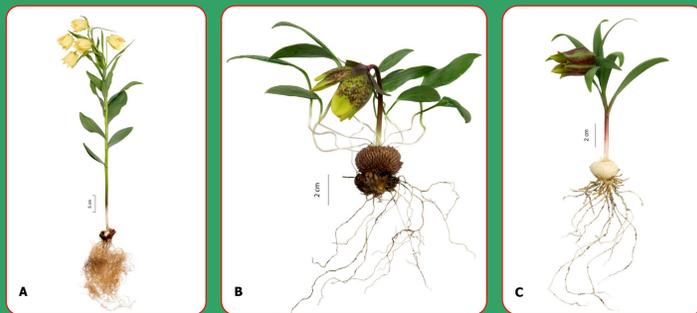


Figure 5. Examples of the range of morphological characters and states to be found within *Fritillaria*. A) *F. pallidiflora* (subgen. *Fritillaria*); B) *F. davidii* (subgen. *Japonica*); C) *F. kotschyana* (subgen. *Fritillaria*)
Photos by L. Hill

References

(1) – Rix, E. M. (2001) *Fritillaria*: A revised classification. *Alpine Garden Society, UK*. (2) – Ransted, N. et al. (2005) *Mol. Phylogenet. Evol.* **35**: 509–527. (3) – Leitch, I. J. et al. (2007) *J. Evol. Biol.* **20**: 2296–2308. (4) Ambrožová, K. et al. (2011) *Ann. Bot.* **107**: 255–268. (5) Swofford, D. L. (2003) PAUP*4.0b10.

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