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Molecular phylogeny, historical biogeography and evolution of truffles

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Objectives

The name “truffle” is a broad term that comprises species of the genus Tuber belonging to the Ascomycota, Pezizales order and Tuberales family. Index Fungorum recognizes 227 species, subspecies and varieties of Tuber, however the truffle classification based on the morphological features of ascomata and spores has led to many controversies. Currently, only 70-75 species have been validated in the world, 32 out of which in Europe. The genus Tuber is only found in the northern hemisphere, between 25° and 60° latitude, spread over diverse climates: from tropical to Mediterranean, temperate, cold-temperate and cold-continental climates.

A combination of different data sets and analyses methods was used as part of a long-standing project aimed at understanding the molecular phylogeny of Tuber; i.e. current distribution patterns of the genus Tuber, phylogenetic reconstruction, dispersal and vicariance analysis and molecular clocks (Wang et al. 2006a,b, Wang et al. 2007 and Jeandroz et al. in press). In addition, we have investigated the truffle evolution and identified genomic regions which could be involved in the speciation events.

Materials and Methods

The phylogenetic relationships among the Tuber species were reconstructed using a dataset of 18S rRNA, 5.8S rRNA, 5.8S-ITS2 rRNA and B-tubulin. Elongation Factor 1 Alpha and Protein Kinase C sequences obtained from species harvested in Asia, Europe and North America. We applied different phylogenetic inference methods: Maximum Parsimony (MP), Bayesian analysis and Neighbour Joining.

We tested the global molecular clock hypothesis for 18S rRNA, 5.8S rRNA, 5.8S-ITS2 rRNA, B-tubulin, EF-1α, 18S+5.8S rRNA and 18S+5.8S+8-tubulin sequences by use of Tajima's relative rate test in MEGA 3.1. These genes, combined or on their own, were employed for molecular clock estimates after construction of linearized trees using MEGA 3.1. We reconstructed ancestral areas with dispersal-vicariance analysis using DIVA v. 1.1 in the Northern Hemisphere. Finally, we used genomic suppression subtractive hybridization (gSSH) to isolate specific sequences of the black truffle T. melanosporum which are absent in the white truffle T. borchii. The obtained clones were sequenced and tested for their specificity through a dot blot experiment.

Results

The molecular phylogeny divided the genus Tuber into five distinct clades (see Figures 1 and 2 in Jeandroz et al. in press). The basal clade I (Aestivum-group sensu lato) contained only European species. It consists of three subclades corresponding to T. macrosporum, T. magnatum and Aestivum-group sensu stricto. The Excavatum-group (Clade II) was represented by the European species, T. excavatum. The Rufum-group (Clade III) was divided in four subclades. Subclade III-1 corresponded to misidentified Chinese individuals, reclassified as Tuber sp. 1 (Wang et al., 2007). Subclades III-2, III-3 and III-4 correspon-
ded, respectively, to the following Chinese species: T. foaeotumense, T. huidongense and T. liyuanense. The grouping of the remaining taxa depended on the phylogenetic methods that were used. In the Bayesian tree, T. rufum and T. terrigneum were grouped into the same subclade. A different topology was observed in the MP tree, but was supported by a low bootstrap value of 50%. The two American species grouped together in the MP tree, while they corresponded to separate lineages in the BI tree.

Clade IV, identified as the Melanosporum-group, consisted of two European species (T. melanosporum and T. brumale) and two Asian species (T. indicum and T. pseudoexcavatum).

Clade V contained the species belonging to the Puberulum-group and was divided into four subclades. Subclade V-1 was formed by one European species, T. scropusum and by one American species, T. whetstonense. Subclade V-2 was formed by three European species, T. foaelidium, T. maculatum and T. rapaeodorum. These two latter species appeared to be paraphyletic. Subclade V-3 was only composed of Chinese species, two misidentified species reclassified as Tuber sp. 3 and Tuber sp. 4 (Wang et al., 2007). Subclade V-4 was comprised of four European species, T. borchii, T. diphylhum, T. oligospermum and T. puberulum, two Chinese species, T. iulii, and one misidentified species reclassified as Tuber sp. 2 (Wang et al., 2007).

Tajima’s relative rate test showed that Tuber 18S rRNA, 5.8S rRNA, 18S rRNA and B-tubulin sequences evolved in a clock-like manner. According to the global molecular clock analysis, the radiation of the genus Tuber could have started between 271 and 140 Mya. Clade I (Aestivum-group sensu lato), clade II (Excavatum-group), clade III (Rufum-group), clade IV (Melanosporum-group) and clade V (Puberulum-group) would have diverged, respectively, between 160 Mya and 140 Mya, 111 Mya ago, 70 Mya, between 85 Mya and 25 Mya and between 65 Mya and 53 Mya.

The DIVA analysis inferred the ancestral distribution from the different nodes and the reconstruction required 9 dispersals. Equally optimal distributions were obtained for several nodes suggesting different possible biogeographical histories. Considering each group separately, the DIVA analysis showed a European ancestral distribution of the ancestors of the Magnatum-, Macrosoron- and Aestivum-groups. Ambiguous ancestral distributions were observed for the ancestors of the four other groups: E or EA for the Excavatum-, Puberulum- and Rufum-groups and E or A for the Melanosporum-group.

Using the gSSH we identified specific genomic fragments from T. melanosporum. Sixty three % of the sequences did not show homology in databases, 16% showed homology with hypothetical protein sequences, 16% showed homology with retropseudoson sequences and 5% showed homology with bacterial sequences.

Conclusion

Molecular phylogeny allowed us to identify several discrepancies with the classical taxonomy of the genus Tuber and to propose a phylogenetically based classification. According to the MP and Bayesian molecular phylogenies (Jeandroz et al., in press), the genus Tuber appeared to be organized in five distinct clades. Although giving slightly different results, the two analyses clearly showed that T. panniferum, previously considered a part of the Rufum-group, was phylogenetically far removed from the Rufum-group, and instead belongs to the Aestivum-group sensu lato. Both phylogenetic analyses also showed that the European species, T. magnatum, until now included in the Puberulum-group, was phylogenetically well-differentiated from the other species of this group and belonged to the Aestivum-group sensu lato.

We also showed that the Macrosoron-group (T. macrosoron, T. foaelidium, T. malençoni, T. pseudoexcavatum and T. regiianum), as defined by Rousset et al. (2001), appeared polyphyletic. At least two species were reclassified into other groups, T. pseudoexcavatum in the Melanosporum-group and T. foaelidium in the Puberulum-group.

According to the different sequences used for molecular clocks, the radiation of the genus Tuber could have started between 271 and 140 Mya. The supercontinent of Pangea would have begun to separate into two masses, the Gondwana and the Laurasia, late in the Triassic Period (245 to 208 Mya) or early in the Jurassic Period (195 Mya). Consequently, we cannot exclude a Pangean origin of the common Tuber ancestor or its presence in only the northern part of the Pangea. During the Cretaceous, the South Atlantic Ocean opened, but North America was still connected to Europe through the North Atlantic Land Bridge. Forty-five million years ago, North America and Greenland split away from Europe and the North Atlantic Bridge was broken. To explain the presence of the genus Tuber both in America and Eurasia, the existence of a migration through the North Atlantic Bridge has to be taken into consideration for the species, which would have diverged before 45 Mya. According to the molecular clock analysis, we estimated the divergence time between the North American species T. quercicolata, T. candidum and T. whetstonense and the European species belonging to the same group, respectively, as 42, 40 and 18 Mya. Consequently, we can roughly estimate that the radiation of these American species occurred after breakdown of the North Atlantic.
Bridge. Nevertheless, given the usually large confidence intervals, dispersal across the North Atlantic Bridge cannot be completely excluded. Another route of terrestrial migration between North America and Eurasia occurred during the Eocene via the Bering Land Connection between Siberia and Alaska. We hypothesize that three groups, the Pseudotuberculatum-group, the Ruflum-group, and perhaps the Melanosporum-group, could have migrated towards North America with their host from the middle of the Miocene via the Bering Land Bridge.

The DIVA ancestral area reconstructions suggested that the common Tuber ancestor originated from the Laurasian region, from what later became Europe or from a larger area, which later became Eurasia. In conjunction with divergence time estimates and probable intra- and inter-continental land connections, we were able to propose two scenarios of intra- and inter-continental diversification of the genus according to the geographic distribution of the more recent common ancestor, Europe or Eurasia. Reconstruction of ancestral distribution areas requires 9 dispersals and the two proposed biogeographical scenarios mainly implied intra-continental dispersal events between Europe (E) and Asia (A) (6/9). Only one dispersal appeared characteristic of each of the two scenarios, while the other dispersals were common to both scenarios. Whatever the hypothesis may be, a first diversification event lead to the Aestivum-Magnatum-Macrosorum- and Patinaferum-groups only being found in Europe. These four groups represent the oldest lineage. However, it is currently impossible to choose between the two hypotheses of the origin of the genus Tuber: Despite the limitations of our current phylogenetic hypothesis (non exhaustive taxa sampling), we were able to show the relative role of dispersal-vicariance events in shaping distribution patterns in the genus Tuber. Dispersal through ancient land connections appeared to have been more important for the biogeography of Tuber rather than vicariance, although the real biogeography was no doubt more complicated.

Finally, we demonstrate that gSSH can be used to identify specific genomic regions between mycorrhizal fungal genomes. In addition, the results suggest that retrotransposons might have played a role in truffle evolution. This hypothesis could soon be verified since the genome of the black truffle Tuber melanosporum will become available in the next months.

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Bibliography


