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Patterns of Speciation and Adaptive Radiation in *Parnassius* Butterflies

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Abstract: The number of species of the genus *Parnassius*, greatly outnumbers those in other genera of the subfamily Parnassiinae, suggests a unique evolutionary history for the Apollo butterflies. Recent extensive molecular analyses evidenced a relatively rapid radiation of *Parnassius* but additive studies are required for a more comprehensive understanding of the evolution of this genus. Then, the relationships between *Parnassius* phylogeny and host-plant preferences have been investigated using molecular phylogenetic analyses. *Parnassius* molecular phylogeny using a combined data set of four mitochondrial genes is congruent with the larval host-plants phylogeny inferred from chloroplast *matK* gene. Phylogenetic analyses evidence that *Parnassius* species have been divided in two groups, the *Parnassius* subgenus which feed mainly on Crassulaceae and all the other subgenera, containing the most basal species, which feed mainly on *Corydalis*. However, these co-analyses alone fail to explain all the observed patterns, indeed if host-plant shifts could explain sympatric speciations, other events, including ecological changes (habitat and elevation) have also play an important role. In conclusion, according us, only a combination of sympatric and allopatric speciations due to numerous factors could explain the *Parnassius* rapid adaptive radiations.

Key words: *Parnassius*, Lepidoptera, adaptive radiation, host shifts, insect-host plant interactions

Introduction

Swallowtail butterflies of the genus *Parnassius* Latreille (1804) are currently used as model organisms in several research areas, including genetics, phylogenetics of host plant utilization and mimicry, mechanisms of speciation and conservation. They also attract both museum taxonomists and amateur collectors because of their geographic variability and rarity of some species that occur in the remote alpine areas of the Himalayas, Central Asia, Tibet and other parts of northern Eurasia (in paleartic mountains). To date, the phylogenetic relationship between *Parnassius* and its related genera remains controversial. According to traditional studies, the genus *Parnassius* is classified with two other genera of similar morphology, *Archon* and *Hypermnestra*, into the tribe Parnassiini of the subfamily Parnassiinae (Munroe, 1961; Hancock, 1983). Together with the tribe Zerynthiini comprising five genera (*Zerynthia*, *Allancastria*, *Sericinus*, *Bhutanitis* and *Luehdorfia*), they are usually regarded as relatively primitive groups in the family Papilionidae (Munroe, 1961; Ackery, 1975; Igarashi, 1979; Hancock, 1983; Häuser, 1993). However, in recent extensive phylogenetic analyses (Omoto *et al*., 2004; Katoh *et al*., 2005) *Parnassius* was found to be most

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closely related to *Hypermnestra helios*, whereas *Archon apollinus* was more closely related to members of the tribe Zerynthiini. In addition, if previous studies have called into the question the monophyly of Parnassiinae (Häuser, 1993; Yagi *et al*., 1999; Caterino *et al*., 2001), recent molecular investigations have evidenced that the genus *Parnassius* constitutes a monophyletic group among the subfamily Parnassiinae, with a number of separate lineages that probably arose through a relatively rapid radiation event during evolution (Omoto *et al*., 2004; Katoh *et al*., 2005). Other genera in the same subfamily are clearly separated from *Parnassius*, suggesting that these genera represent evolutionary much older lineages. In addition, the number of species of the genus *Parnassius* which is greatly outnumbers those in other genera of the subfamily Parnassiinae, suggesting a unique evolutionary history for the *Parnassius* genus (~50 species on 69 divide in 8 genera). Within the genus *Parnassius* up to 10 species-groups or subgenera have been proposed based on morphological and behavioural studies (Bryk, 1935; Eisner, 1958, 1968; Munroe, 1961; Ackery, 1975; Hancock, 1983; Weiss, 1992, 1999). The exact number of species, however, is not known because of disagreements as to the species/subspecies rank. The morphological characteristics primarily used in the classification of species and species groups include wing pattern, venation, male genitalia, fore-tibial epiphysis and sphragis, i.e., the attachment to the end of the female abdomen made by the male secretion during copulation (Hancock, 1983). On the basis of morphological comparisons, it has been suggested that butterflies of the genus *Parnassius* had undergone much speciation in the Tibetan region since early Tertiary period (Hancock, 1983). However, the biological relationships among species and species-groups, as well as the evolutionary scenario of *Parnassius* butterflies have thus far only been speculative. In addition, despite extensive molecular analyses, the phylogenetic relationships among *Parnassius* sub-groups were not clear (Omoto *et al*., 2004; Katoh *et al*., 2005). Therefore, complimentary studies are required to elucidate the relationships within this genus. The number of species of the genus *Parnassius* (more than 70% of the subfamily), suggesting rapid adaptive radiations added to the host-plants shifts is ideal for co-analysis of animal and plant phylogenies. Indeed, it is well known that adaptation to alternate host plants can cause reproductive isolation and speciation (Dre`s and Mallet, 2002). In this study, relationships between *Parnassius* phylogeny using a combined data set of four mitochondrial genes and host-plant phylogeny inferred from chloroplast *matK* gene have been investigated.

Materials and Methods

Butterfly Taxon Sampling

The species of Parnassiinae butterflies and host-plants investigated in this study are listed in Table 1. Our choice of butterfly specimens to be analyzed was guided by the necessity to include at least one species of the major potential subgenera of the *Parnassius* genus (Table 1). They have been collected in broad areas of Eurasia.

DNA Extraction, PCR Amplification and Sequencing

All the chloroplast *matK* nucleotidic sequences of host-plants were extracted from GenBank. For the *Parnassius* analyses, DNA was extracted from legs or heads of dried adult butterfly according to a method described by Monte-Alegre *et al*. (2005). Three regions of the mitochondrial genome were amplified by Polymerase Chain Reaction (PCR) using the following oligonucleotide primers: 5'-CGCCTGTTTATCAAAAACAT and 5'-CCGGTTTGAGCTCAGATCA for the *LSU*(16S rRNA) gene (Simon *et al*., 1994); 5'-CGTAAAGTCCTAGGTTATATTCAGATTCG

Table 1: Life history characters for *Parnassius* species and GenBank accession numbers for butterflies and host-plants analyzed in this study

Parnassius subgenera[1]	Species	Distribution[2]	Locality[3]	GenBank accession No.				Larval food plants[4]	GenBank accession No. with the species
				COI	*ND1*	*ND5*	*16S*		*matK*
Driopa (Corydalis)	*P. mnemosyne* (Linnaeus, 1758)	W Europe, c. Asia	France (Lachens)	CR[5]	CR	AB095626	CR	*Corydalis*	Fumariaceae : AF543734 *Dicentra eximia*
Kailasius (Corydalis Fumaria)	*P. charltonius* (Gray, 1852)	W c. Asia	Pakistan (Satpara pass)	CR	CR	AB095630	CR	*Corydalis*	idem
	P. imperator (Oberthür, 1883)	Tibet, E China	Chine (Gansu Qilianshan)	CR	AB186191	AB095612	CR	*Corydalis*	idem
Koramius (Corydalis Cysticorydalis)	*P. acdetis* (Grum-Grshimailo, 1891)	Karakorum, E China	Pakistan (Lakul)	CR	AB186191	AB095612	CR	*Corydalis*	idem
	P. delphius (Eversmann, 1843)	Tian Shan	Kyrgyzstan (Dolon)	CR	AB186190	AB095621	CR	*Corydalis Cysticorydalis*	idem
	P. staudingeri (Bang-Haas, 1882)	Pamir, Karakorum, Hindukush	Kyrgyzstan (Aram Kunghei)	CR	CR	AB095637	CR	*Corydalis Cysticorydalis*	idem
Tadumia (Corydalis)	*P. hardwickii* (Gray, 1831)	Kashmir, S Tibet	Pakistan (Deosai)	CR	AB186178	AB094969	CR	*Corydalis* Saxifragaceae	AF133136 *Saxifraga caesia*
Parnassius (Crassulaceae Saxifraga)	*P. phoebus* (Fabricius, 1793)	Alps, Sakhalin	France (Hautes-Alpes)	CR	CR	AB095654	CR	Saxifraga Crassulaceae	idem AF115667 *Sedum rupestre*
	P. actius (Eversmann, 1843)	T-Shan, W China	Kyrgyzstan (Dolon)	CR	CR	AB095622	CR	Crassulaceae	idem
	P. apollo (Linnaeus, 1758)	Europe, Altai	Greece (Peloponnese)	CR	CR	AB095636		Crassulaceae	idem
	P. epaphus (Oberthür, 1879)	Karakorum, Qingai	Pakistan (Satpara pass)	CR	AB186176	AB095610	CR	Crassulaceae	idem
	P. jaquemontii (Boisduval, 1836)	Tian Shan, W China	Kyrgyzstan (Tenghisbay)	CR	CR	AB095647	CR	Crassulaceae	idem
	P. nomion (Fischer von Waldheim, 1823)	Urals, Korea	Russia (Buriatia)	CR	AB186175	AB095609	CR	Crassulaceae	idem
	P. tianschanicus (Oberthür, 1879)	W c. Asia	Kyrgyzstan (Dolon)	CR	CR	AB095648	CR	Crassulaceae	idem
	P. apollonius (Eversmann, 1804)	Tian Shan	Kyrgyzstan (Susamyr valley)	CR	CR	AB095646	CR	Crassulaceae Salsola *Scabiosa*	idem AY514843 *Salsola kali* AF446918 *Scabiosacolumbaria*
Sachaia (Lagotis Corydalis)	*P. simo simonius* (Staudinger, 1889)	Alai	Kyrgyzstan (Tenghisbay)	CR	CR	AB095649	CR	*Lagotis*	Plantaginaceae: AY667458 *Bacopa monnieri*
Tribe *Zerynthini*	*Zerynthia rumina* (Linnaeus, 1758)	S Europe (Basses-Alpes)	France	CR	AB186201	AB095660	CR	*Aristolochia*	AF543724 *Aristolochia pistolochia*

[1]With the commonly used host plants in parentheses. [2]Geographical ranges are abbreviated as follows: c., central; E, East; S, South; W, West. [3]Localities only for the samples sequenced in our laboratory. [4]Sources: (Seppänen, 1970; D'Abrera, 1990; Chou, 1994; Tuzov *et al*., 1997; Tolman and Lewington, 1997). [5]CR: Sequences submitted to GenBank.

and 5'-ATCAAAAGGAGCTCGATTAGTTTC for the *ND1* gene (Aubert *et al*., 1996) and 5'-GGTCAACAAATCATAAAGATATTGG and 5'-TAAACTTCAGGGTGACCAAAAAATCA for the *COI* gene (Folmer *et al*., 1994). PCR components per 50 µL reaction were as follows 50 ng template DNA, 0.2 µM of each primer, 2.0 U. HiTaq *Taq* polymerase, 0.2 mM dNTPs, 5 µL of the reaction buffer provided by the *Taq* manufacturer (Bioprobe, France). The cycling parameters were as follows 92°C for 2 min, 5 times (92°C for 40 sec 43°C for 45 sec and 62°C for 1.5 min), 30 times (92°C for 40 sec 47°C for 45 sec and 62°C for 1.5 min) and 62°C for 8 min. Using the single-stranded DNA as a template, the nucleotide sequence was determined with an automated DNA sequencer (Genome Express, Grenoble, France, http://www.genomex.com). All nucleotide sequences analyzed in this paper have been deposited in the GenBank. In addition, some Parnassiinae sequences have been extracted from GenBank (all the *ND5* gene sequences, the four mitochondrial genes of the outgroups and due to problems with amplifying, seven *ND1* gene sequences of *Parnassius*).

Phylogenetic Analyses

Sequences were aligned using Clustal W software (Thompson *et al*., 1994). Phylogenetic analyses were performed in PHYLIP version 3.6 alpha 3 (Felsenstein, 2002) accessed at http://www.infobiogen.fr/. Three different approaches have been used : 1) the Neighbor-Joining (NJ) method (Saitou and Nei, 1987); 2) a cladistic approach using the Maximum Parsimony (MP) criterion (Swofford, 1998); 3) Maximum Likelihood (MLH) reconstruction was performed using estimations of all model parameters from the data set. Robustness of nodes was estimated by running a bootstrap test with 100 replicates for NJ, MP and MLH trees.

Results

Parnassius Genus Phylogenetic Analysis

In the *LSU* region, alignment gaps were observed at several nucleotide sites. Therefore, these sites were excluded and the remaining 488 sites were used for further analyses. In contrast, no alignment gaps were observed in the three protein coding genes (*COI*, *ND1* and *ND5*), such that the consecutive 615 sites, 455 sites and 725 sites could be used, respectively for further analyses. In order to check that there was no bias introduced by one of genes, all the partial gene sequences were analysed separately using three phylogenetic methods (unweighted Maximum Parsimony (MP), Neighbor Joining (NJ) and Maximum likehood (MLH)). In addition, in these various preliminary tests, numerous *Parnassiinae* sequences (*Battus philenor*, *Iphlictides podalirius*, *Papilio machaon*, *Zerynthia rumina*) available from GenBank were selected in turn or together as outgroups. In all trees obtained with sequences of these four species, the *Parnassius* genus formed a monophyletic group. Using a largest dataset and, respectively *ND5* genes alone or both *ND5* and *LSU* sequences, Omoto *et al*. (2004) and Katoh *et al*. (2005) have already evidenced the monophyly of this genus. In addition, alignments that contained any single outgroup taxon, or a combination of any four outgroup taxa, did not generate great variation of tree topology among the ingroup taxa for any of the algorithms that produced unrooted trees. In preliminary studies, each gene has been used separately. All the molecular phylogenetic trees obtained by the three different methods show essentially the same branching pattern and in some portions of the tree where results were varied for different methods, the bootstrap values were low (data not shown). As there was not serious incompatibility of datasets generated from the four DNA sections, we carried out a combined analysis in which 68 sequences (17 for each gene) were used, the four alignments (one by DNA partial genes) were aligned together (final alignment is available from the authors upon request).

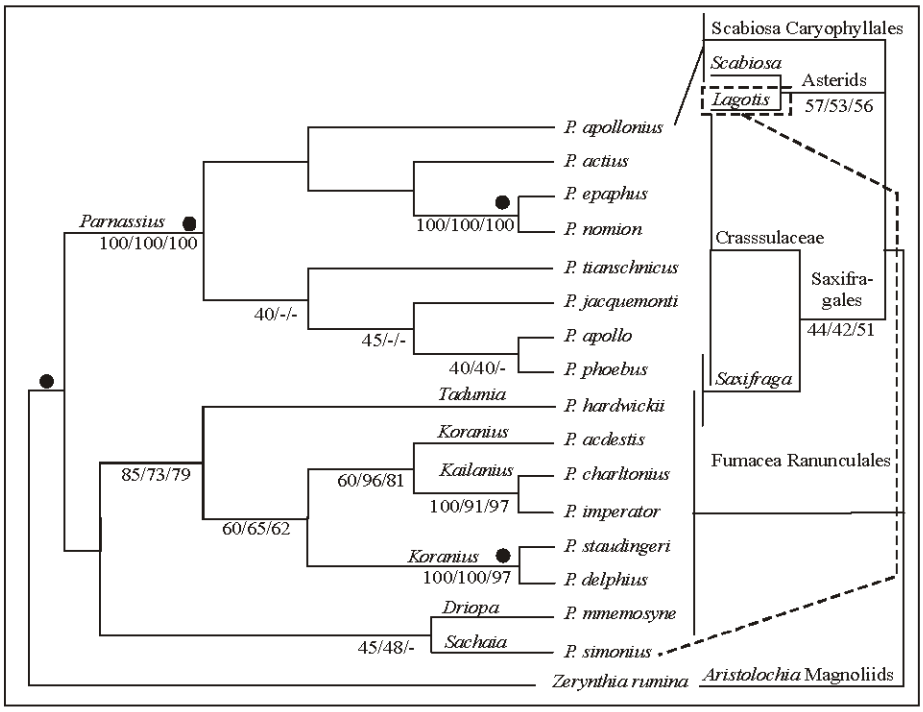

Fig. 1: Comparison of phylogeny of *Parnassius* species with their host-plants phylogeny. *Parnassius* phylogeny has been inferred from concatenated sequences (*COI*, *ND1*, *ND5* and *LSU*), partial *matK* genes has been used for plant phylogeny. As the trees are not significantly different in topology so only the Maximum Parsimony trees are shown, the branch lengths are approximate. Bootstrap values (100 replicates) are shown below branches, Maximum Parsimony (left bootstrap value), Neighbor Joining (middle bootstrap value) and Maximum likelihood (right bootstrap value), only selected values over 39 are given. In the *Parnassius* tree, a black circle denotes that this node has been found when the four data sets have been analysed separately, an asterisk (*) denotes that a similar topology has been found in the Omoto *et al.* (2004) analysis

Rooting on *Z. rumina* species, the three tree-making methods (MP, NJ and MLH) produced similar topologies, which displayed one weakly supported group and one strongly supported clade corresponding to the *Parnassius* subgenus (Fig. 1). In the first group, two subgroups were apparent, one of them containing two species belonging to two different subgenera (*Driopa* and *Sachaia*) was not statistically supported and this branching patterns was unstable, the last one contained species belonging to three subgenera (*Tadumia*, *Koramius* and *Kailasius*) and was well supported by bootstrap analyses (BP = 85/73/79). In this last clade, *Tadumia* emerged as a sister taxon to the two other subgenera and *Koramius* was paraphyletic but this was not supported statistically.

Interestingly, the higher bootstrap values were found in clades which exhibited similarly high bootstrap values in phylogenetic analyses inferred by one of the four genes alone (*Parnassius* subgenus, *P. epaphus* + *P. nomion* and *P. staudingeri* + *P. delphius*), in addition, the

phylogenetic analyses of Omoto *et al.* (2004), in spite of the fact that their datasets were more complete, exhibited similar groupings. In addition, other groupings which were in agreement with this previously published study are shown in Fig. 1. In addition, similarly to Omoto *et al.* (2004) and in spite of the fact that our data set contains only two species - but one by sub-group according to Wiess (1999) - our analyses confirm the monophyly of the *Kailasius* subgenus. This taxon is composed of seven species with wide distribution ranging from Central Asia through the Great Himalayas to western China. They are mostly big and brilliantly colored butterflies and usually classified under two different species-groups morphologically distinguished, viz., the *Charltonius* group and the *Imperator* group. Moreover, our analyses also evidenced that *P. acdestis,* which belongs to *Koramius* subgenera, is found rather to belong to this cluster or at least to be a sister group. In the molecular analyses of Omoto *et al.* (2004) and Katoh *et al.* (2005), *P. acdestis* was also found to belong to the *Kailasius* cluster.

Host-plants Phylogenetic Analysis

Parnassius species of our representative data set make use together in the wild of no less than seven different plant families that belong to several of the major clades of angiosperms. All the *Parnassius* host-plant species have not been sequenced, when no sequence of the corresponding genus has been found in GenBank, homologous sequence of the nearest taxon has been chosen. Concerning the host-plants phylogeny, the *matK* gene has been chosen because it has been evidenced that this gene provides good resolution within many angiosperm sub-taxa (Hilu *et al*., 2003). Our phylogenetic analysis of 914 unambiguous sites (Fig. 1), of which sampling is too weak, does not claim to reflect the phylogeny of magnoliophyta, however, its topology is not in conflict with other molecular analyses. Numerous molecular studies have evidenced that both magnoliids and eudicots are resolved as monophyletic lineages (Borsch *et al*., 2005; Löhne and Borsch, 2005). Within eudicots, which comprise about 75% of angiosperm species diversity, many phylogenetic questions persist (e.g., Soltis *et al*., 2000; Judd and Olmstead, 2004), for example, the position of ranunculales (Fumacea in our analysis) could differ, being either basal in eudicots or sister to a eudicot clade. In addition, if the monophyly of the core eudicot clade is not ambiguous, relationships among the major clades of this last taxon (three lineages in our dataset : saxifragales, caryophyllales and asterids) remain uncertain (Hilu *et al*., 2003).

Discussion

Relationships Between Parnassius Phylogeny and Host-plant Utilization

In the ongoing debate about the extent of congruence to be expected between the phylogenies of insects and their host plants, butterflies of the Papilionidae family have occupied a central place ever since 1964, when Ehrlich and Raven (1964) published their seminal paper on the proposed coevolution of butterflies and plants. If only for this reason, there have been continuing and unusual efforts to collect as much information as possible on host plant utilization by swallowtail larvae.

Butterflies of the genus *Parnassius* are known to utilize host plants belonging to eight families, although most host-plant records are from two families (Seppänen, 1970; D'Abrera, 1990; Chou, 1994; Tuzov *et al*., 1997; Tolman and Lewington, 1997). Table 1 underlines that *Parnassius* are associated principally with two groups of plants: Fumariaceae-mainly *Corydalis*-and Crassulaceae-*Rhodiola*, *Sedum*, *Sempervivum* and related genera. In our phylogenetic analysis, the *Parnassius* species have been divided in two groups of which the commonly used host-plants are

different, the Saxifragales (mainly Crassulaceae) for the *Parnassius* subgenus and Fumariaceae and/or *Lagotis* for the other subgenera. *Parnassius* species which feed on a high altitude Scrophulariaceae *(Lagotis)* constitute a localized and interesting exception concerning only species of the *Simo* group which belongs to the *Sachaia* subgenus (Weiss, 1999).

Interestingly, in our data set, two species which emerged as sister group to many others (*P. apollonius*/other *Parnassius* species of this subgenus and *P. harwickii*/species belonging to *Koramius* and *Kailasius* subgenera) have larvae which could consume host-plants belonging to two different plant orders. In their NJ tree based on the *ND5* sequence, Omoto *et al.* (2004) have evidenced similar topologies, *P. apollonius* (with *P. honrathi*) is the sister group of the other species of the *Parnassius* subgenus and *P. harwickii* is the sister group of all the *Parnassius* species except them belonging to the *Parnassius* subgenus. However, these topologies in the two studies have not supported statistically.

The Ancestral Parnassius Host-plant and the Various Shifts

Is it possible to guess at ancestral host plant utilization in *Parnassius* species? Obviously, the original food plants of the Parnassiinae-Zerynthiinae phylum are the Aristolochiaceae, still used by *Archon* and all Zerynthiinae. The sister genus of *Parnassius*, *Hypermnestra*, lives on Zygophyllaceae plants (*Zygophyllum*, *Halimiphyllum*) from the arid regions of western central Asia (D'Abrera, 1990; Tuzov *et al*., 1997). It is thus likely that the stem common to both lines had already left the Aristolochiaceae. Interestingly, *Parnassius glacialis* Butler, 1866 (*Driopa* subgenus, *mnemosyne* group) whose principal host-plants are *Corydalis* still feed on *Aristochia* (Chou, 1994). This parnassian species inhabits arctic or mountainous area of the Northern Hemisphere and has morphological characteristics that were suggested to be primitive (Nemoto and Inomata, 1994; Weiss, 1999).

The most basal species of *Parnassius* in the phylogenetic trees of the extensive studies of Omoto *et al.* (2004) and Katoh *et al.* (2005) are linked to *Corydalis*, a genus which contains about 320 species, mainly localized in central Asia (Heywood, 2005). Two other elements are in favour of this hypothesis, in one hand, most of the species of five on six subgenera feed mainly on plants of this genus and in another hand, the feed choice of *P. glacialis* evidenced probably the first ancestral plant shift.

Inside of *Parnassius*, the major foodplant change concerns the *apollo* group (*Parnassius* subgenus), which have adopted another group of plants adapted to mountains, the Crassulaceae. These host plants are a morphologically diverse and systematically complex angiosperm family comprising 35 genera and 1500 species (Berger, 1930). The family inhabits primarily semiarid and mountainous habitats and even if now the distribution is nearly cosmopolitan, most of the species still exhibit xerophytic adaptations (e.g., succulent leaves, a thick waxy cuticle and crassulacean acid metabolism). Therefore, the second major change has been performed inside of the same habitat allowing sympatric speciations.

The same is observed with a more restricted shift, that from *Corydalis* to *Lagotis* in the *simo* group of the subgenus *Sachaia*-the other species of this subgenus remains linked to *Corydalis* (Korshunov and Gorbunov, 1995): *Lagotis* lives on high elevation screes, where certain species of *Corydalis* happen to be numerous. This is true also for the shift of *phoebus* to the widespread *Saxifraga* like *S. aizoides* in the Alps: this plant lives in exactly the same habitat as the supposedly genuine foodplant, *Rhodiola rosea*, the shores of torrents. Interestingly, this last shift within species which belong to the same plant order (saxifragales) and certain populations of the species still feed on Crassulaceae like *Sedum* or *Rhodiola* species.

Is Chemistry Could Unit All the Parnassius Host-plants

A number of aposematic butterfly sequester unpalatable or toxic substances from their host plants rather than manufacturing their own defensive substances (Nishida, 2002). One type of toxic compounds among the most used by butterflies is alkaloid. Numerous alkaloids have been isolated in species belonging to all the *Parnassius* host-plants genera (Willaman and Schubert, 1961; Israilov *et al*., 1984; Zhu, 1998; Seger *et al*., 2004; Zhao and Ding, 2004; Endonova and Antsupova, 2005; Li *et al*., 2005) except for Crassulaceae. However, if in this family, all the genera have not been chemically investigated and these toxic compounds have been found at least in *Sedum* and *Rhodiola* species (Steven *et al*., 1995; Tolonen, 2003).

The *Corydalis* genus is closely related to the Papaveraceae, they are toxic and contain numerous alkaloïds. *Parnassius* have a wing pattern suggestive of aposematism and they display their eyespots with stridulation when disturbed at rest (Descimon, 1965); the larvae can be mimetic of toxic millipedes (Scott, 1986; Deschamps-Cottin and Descimon, 1996). It is therefore likely that *Parnassius* store toxic compounds produced by *Corydalis*.

Crassulaceae, the other preferred host plants, also contain alkaloids and can provide protective compounds as well, for example, piperidine alkaloids are widely distributed in *Sedum* species (Hegnauer, 1989). In addition, it is known that sarmentosin, a bitter-tasting cyanoglycoside produced by Crassulaceae that is not cyanogenic itself, occurs in *Parnassius* butterflies (Nishida and Rothschild, 1995). These cyanophilic insects apparently show host-exploitation patterns based on innate resistance to the toxins (Nishida, 2002).

The picture of *Parnassius* host-plants is somewhat complicated by the fact that data concerning their chemical characteristics are very partial, for example, it would be interesting to know if a (secondary) chemical product, if it exists, units the *Parnassius* host-plant families. We hypothesise that further extensive chemical investigations could give interesting results. For example, all the *Euphydryas* host-plants, which belong to 13 different families are united by the presence of a group of secondary defense chemicals known as iridoids (Jensen *et al*., 1975). Both are known to be extremely and Bowers and Puttick (1986) have shown that iridoid glycosides are necessary feeding stimulants for Nearctic *Euphydryas* larvae and that the larvae are able to sequester these compounds for use in their own chemical defense (Zimmermannn *et al*., 2000). *Euphydryas* larvae are considered aposematic and they are unpalatable to birds (Bowers 1981). Interestingly, iridoid glucosides have been found in *Lagotis* (Yang *et al*., 2004) and in Crassulaceae (Tolonen, 2003) and a putative precursor of seco-iridoid has been detected in *Scabiosa* (Horn *et al*., 2001). However, to date, it is not known, if some *Parnassius* species were able to sequester these compounds.

In addition, chemoreception is an essential function in the recognition of host plants for phytophagous insects. Detection of a specific repertoire of plant secondary metabolites by olfaction or contact chemoreception plays an important role in determining whether or not a plant is suitable (Nishida, 1995; Ono and Yoshikawa, 2004). And we cannot exclude phytochemical mediators serving as attractants, repellents, stimulants, or deterrents in oviposition behavior of *Parnassius* could units their host-plants.

Important Role of Ecological Factors (Habitat and Elevation) in Parnassius Evolution

According to Hancock (1983) the center of diversity of the Parnassius is in the Central Asian high plateau and the time of first divergence. The adaptation to high elevation habitats seems to have occurred at the second step of *Parnassius* evolution : the most basal branch (*Dryopa*) is, in its generality, associated with low mountain, semi-forested biomass. In central Asia, habitats from

mountain zone clearings to the highest screes, ridges and slopes up to snow line are colonized by at least one species of *Parnassius* (and in Ladakh, up to seven species can be observed around Nimaling, 4000-5500 m : Weiss, 1992). This adaptation to higher elevation could explain that Glacial periods are likely to have been favourable to the extension of *Parnassius*, hence the wide distribution of the *P. Apollo* species, which reached the mountains of Andalucia as well as the Etna in Sicily and Taygetos mountain in southern Greece.

Conclusions

Judging from the branching pattern of their phylogenetic trees, which exhibit that the *Parnassius* clades appeared to be connected to each other by relatively short internal branches, Omoto *et al.* (2004) and Katoh *et al.* (2005) have suggested that the genus *Parnassius* would seem to have originated by a relatively rapid radiation. According to these authors, the time of the rapid radiation took place sometime in the late Tertiary period (20-30 MYA BP). It has been proposed (Mitter and Farrell, 1991) that the diversity of plants, in particular their chemical diversity, could be a factor involved in the strong diversification of herbivorous insects. According to what could be called the 'explosive adaptive radiation theory', a 'key' character evolves in a lineage that enables it to explore new niches. The availability of new resources as well as new areas to colonize, with other selection pressures and release from competition, could promote speciation processes (Futuyma, 1986). As soon as an insect has been able to overcome chemical defenses of plants, an opportunity for diversification can occur (Mitter *et al*., 1988). One of the parsimonious strategies for the phytophagous insects is shifts within plants which produced similar secondary toxic compounds, for example, the evolutionary history of host-plant use in the tribe Melitaeini evidenced that plant chemistry is a more conservative trait than plant taxonomy (Wahlberg, 2001). However, the host-plants shifts alone could not explain the relatively rapid radiation event during *Parnassius* evolution. Some ecological traits as habitat and elevational changes are probably also play an important role. According us, only a combination of sympatric and allopatric speciations due to numerous factors could be explain these rapid radiation events. This study evidence the two future areas of research which are required for a more comprehensive understanding of *Parnassius* evolution: first, extensive chemical investigations of the *Parnassius* host-plants; second, analyses of all the ecological factors in a historical perspective.

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