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Pollination ecology provides some new insight into evolution and systematics of Mediterranean Legumes

Abstract

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Plant evolution and diversification is driven by several environmental selective pressures and constraints. Pollinators' ethology, form, and size play a relevant role in determining floral characters, and more so when pollination is specialized (i.e. due to one or very few insect species). Floral characters, in turn, constitute the main ground of traditional classification system. Recent molecular phylogenies of Mediterranean Legumes have challenged the commonly accepted systematics, as several genera usually regarded as "natural" ones proved to be polyphyletic.
The question arises, whether the incongruence between natural groups based on floral traits and monophyla based on molecular homology is due to experimental flaws, or whether it has a biological background. The authors present recent evidence that pollination in Mediterranean Legumes is much more specialized than previously reported. Even species pollinated or visited by several insect species are specialized, in that their fitness is enhanced only by one or few of them, while other visitors and pollinators may exert little influence on seed production, or even depress it. Such specialization may account for homoplasy of floral characteristics, so that several "natural" genera may reflect common pollination syndromes rather than represent monophyletic clades.

Introduction

A great diversity of *Fabaceae* belonging to the subfamily Faboideae is found in the Mediterranean region, where 17 tribes, with about 80 genera and 1800 species are recorded (Greuter & al. 1989), including many endemic and rare taxa. Among tribes having their main diversity centre in the Mediterranean area, let's mention *Loteae* DC., *Coronilleae* (Adans.) Boiss., *Vicieae* (Adans.) DC., *Cytiseae* Bercht. & Presl, the last being by far the richest in species.

It is well known that tribes and genera have been established and defined prevalently on morphological grounds, with a predominance of floral characters (see e.g. Gibbs 1966, 1967; Gibbs & Dingwall 1971; Polhill 1976; Cantó & al. 1997; Polhill & Raven 1981; Talavera & Salgueiro 1999; Talavera & al. 1999; Cristofolini & Conte 2002).

Recent taxonomic revisions of such tribes, predominantly based upon molecular characters, have challenged the traditional taxonomic treatment, as several genera that used to be regarded as "natural" ones, came out to be polyphyletic: this is the case with *Genista* L. (Pardo & al. 2004; Rega & al. unpubl.), *Argyrolobium* Eckl. & Zeyh. (Rega & al. unpubl.), but also with *Teline* Medik. (Pardo & al. 2004; Rega & al. unpubl.), whose morphological uniformity is obvious, and even with *Cytisus* L. sect. *Tubocytisus* DC. (Käss & Wink 1995, 1997), just to mention a few cases.

The inconsistency between "traditional" (morphological) taxonomy and phylogenetic (molecular) relationships is a major point of concern in pursuing a natural system of classification. The issue we want to address here is the following: is the incongruence between molecular and morphological taxonomy due to incomplete knowledge or inappropriate research methods, or does it reflect independent evolutionary patterns?

Pollination: specialization vs. generalization

How specialization and respectively generalization can influence and mould floral traits has been debated at large for many years (for a general overview, see Waser & Ollerton 2006). Even an unambiguous definition of both terms is lacking. As Johnson & Steiner (2000) pointed out, the dichotomy between generalization and specialization in pollination systems is a simplification of what is more likely a continuum and, furthermore, spatial and temporal factors strongly affect the degree of ''ecological specialization'' (Petanidou & Potts 2006). Functional groups of pollinators may contain few or many species, varying in their effectiveness, thus plant species that appear to be generalists at a first sight, may prove to be much more specialized than a simple study of their pollinator spectrum would suggest (Ollerton 1996; Kay & Schemske 2004; Moeller 2005).

We shall take here as an Occam's razor Stebbin's (1970) "most effective pollinator principle", that states that "… the characteristics of the flower will be moulded by those pollinators that visit it most frequently and effectively in the region where it is evolving." A corollary of this definition is that "specialization" does not imply necessarily "pollination operated by only one insect species", but rather "pollination most effectively operated by one or more insect species with similar ethological, structural and dimensional characteristics".

As a further corollary of the "most effective pollinator principle", we expect that a flower most effectively pollinated by one or more insect species with similar ethological, structural and dimensional characteristics ("specialist" flower) will be subject to a very restrictive selective pressure, the more stringent the more homogeneous is the effective pollinators' array, regardless of the number and characteristics of visitor species, and of less effective pollinators.

The vast majority of angiosperms are supposed to be generalists in their pollination requirements, based on the observation that flowers often attract a broad spectrum of visitors (Herrera 1987; Ollerton 1996; Waser & al. 1996; Petanidou & Lambron 2005).

To address this issue, though, it is required not only to distinguish between true pollen vectors and non pollinating visitors, but also to evaluate their pollinator effectiveness. Effectiveness in turn can be indirectly estimated by analysing the pollen carried by visitors (Tepedino & al. 1999; Schlindwein & Medeiros 2006; Galloni & al. 2008). The examination of pollen on visitors' bodies gives an estimation of visitor's fidelity; combining the estimate of fidelity with the estimate of the insect's relative abundance as pollinator provides a measure of "pollinator importance" (Olsen 1997; Larsson 2005; Moeller 2005).

Despite the common evolutionary specialization of their papilionaceous flower, associated to the so-called "bee-syndrome" (Faegri & van der Pijl 1971), legume species show different degrees of ecological specialization of the pollinating system. The taxonomic diversity of pollinators alone cannot be a reliable indicator of the degree of specialization (Waser & al. 1996). Papilionaceous flower obviously reflects the so-called bee-syndrome, i.e. a complex of features that make it fit to hymenopterans pollinators. Nevertheless, most species studied have been indicated as generalists, since long arrays of visitors and/or pollinators have been recorded on them (Rodriguez-Riaño & al. 1999, Galloni & Cristofolini 2003).

So, the question that demands an answer is: are most legumes really "generalist" ? If not, may their specialization account for some degree of convergence in floral evolution? Consequently, may floral similarity in different groups of Legume species be the outcome of homoplasy?

Recent attainments in Legume pollination ecology

1. *Primary versus secondary pollen presentation*.

A survey over more than 30 Mediterranean Legume species representative of 20 genera and 8 tribes (Galloni & al. 2007) revealed that all species examined, except *Hedysarum coronarium* L. and *Onobrychis viciifolia* Scop. (both belonging to tribe *Hedysareae* DC.) are characterized by secondary pollen presentation. In tribes *Cytiseae* Bercht. & Presl, *Vicieae* (Adans.) DC., *Trifolieae* (Bronn) Benth., *Loteae* DC., *Coronilleae* (Adans.) Boiss., *Robinieae* (Benth.) Hutch. and *Galegeae* (Bronn) Torrey & Gray, pollen is usually released while the flower is in the bud stage; corolla, style and filaments are the main structures involved in secondary pollen presentation. This finding confirms literature data (Yeo 1993; Rodriguez-Riaño & al. 1999). Pollen/ovule ratio was higher in species with primary pollen presentation, and lower in species with secondary pollen presentation. Pollen/ovule ratio is an index of pollination efficiency, since less efficient pollen transfer requires higher number of pollen grains compared to ovules to be fertilized. In conclusion, secondary pollen presentation seems to enhance fitness.

It is remarkable that secondary pollen presentation implies that the flower as a whole is involved in the plant-insect interaction, so that fitness is related to the overall floral structure.

2. *Visitors' and pollinators' diversity negatively affects plant fitness*

An analysis of the spectrum of visitors of seven Mediterranean Legume species was performed by Galloni & al.(2009); the potential pollinators were identified by observing insects' behaviour on flowers. The diversity of the assemblage of insects visiting each plant population (flower visitors and potential pollinators) was calculated in order to estimate to which degree pollination systems are specialized. The importance value (PI) for main taxa of potential pollinators was computed considering both insect fidelity and relative abundance. The authors could show that the values of importance and dominance of the pollinator assemblages of the different Legume species are very diverse, revealing various degrees of plant-pollinator specificity despite common papilionaceous floral traits.

Considering only main broader taxonomic categories and relative visitor abundance, the proportion of non-pollinating individuals ranges from very high percentage (*Spartium junceum* L.) to almost none (*Hedysarum coronarium* L.). Fruit production resulted negatively correlated to visitors' taxonomic diversity. In particular, the results showed that the presence of non-pollen vectors was negatively correlated to fruit set, suggesting that non-pollinating visitors could exert a negative effect on successful pollination, possibly disturbing active vectors.

The pollination system of *Cytisus scoparius* (L.) Link is relatively specialized: in the studied population flowers were visited by very few pollinator taxa, among which only *Bombus* species were able to trigger the floral explosive mechanism. A peculiar situation characterizes *S. junceum*: almost only big *Xylocopa* bees can provoke flowers' opening by explosive mechanism, denoting a high level of specificity; however, the most abundant insects observed touching the reproductive flower parts were coleopterans that might accidentally contribute to pollen transfer, despite their commonly known pollen-eating habit (Faegri & Van der Pijl 1971; Woitke & al. 2006). Several species of beetles have been observed also on *Genista radiata* Scop., but in this case their abundance was very low compared to that of main pollinators (*Bombus* spp.). So, in spite of the fact that the flowers are visited by several potential pollinators, their relative abundance indicates some degree of specialization. An analogous condition was observed by *Genista cilentina* Vals., *H. coronarium* and *C. scoparius*, where only one pollinator was usually prevailing (Galloni & al. 2009).

A negative linear relationship was found between the diversity of potential pollinators and fruit set, indicating that a higher pollinator diversity effects a decrease of plant productivity, considering either the number of taxa or the taxonomic distribution assessed with the Shannon index (Galloni & al. 2009). The same authors could show that various degrees of specialization were recognized taking into account the quality of pollination service: the "importance values" (PI) obtained considering both insect fidelity and relative abundance showed differential pollinating ability. Absolute maximum values (PI_{max}) are very dissimilar: *Apis mellifera* for *H. coronarium* and *Bombus* spp. for *C. scoparius* showed the highest values, while the most important pollinators of *Cytisophyllum sessilifolium* O.Lang and *S. junceum* (*Osmia* and *Xylocopa* spp., respectively) showed the lowest ones. Moreover, the indexes of dominance for pollinator importance considerably varied among pollinator assemblages: in *C. scoparius*, *H. coronarium* and *G. radiata* one pollinator clearly dominated over the rest while it was much less evident in *C. sessilifolium*, for which *Osmia* and *Bombus* species showed similar importance values.

The positive correlation between PI_{max} and fruit production demonstrates that reproductive output is promoted by a greater pollinator importance. The negative correlations observed between pollinator and visitor diversity, on the one side, and fruiting success, on the other, together with the positive correlation between maximum pollinator importance and fruit set, point to a positive relation between plant–pollinator specificity and plant reproductive success.

3. Pollinator size affects the plant fitness.

Vivarelli & al. (2011) studied eight populations of *Ononis masquillierii* Bertol., a species endemic to the Northern Apennines. *O. masquillierii* is similar to many other

Mediterranean legumes described e.g. by Free (1993), Pérez-Bañon & al. (2003, and references therein): despite the fact that it received visits exclusively from a restricted phylogenetic group of pollinators (Hymenoptera Apoidea), a 18-fold variation in pollinator body size was observed. Therefore, the complex and specialized flower suggests the possibility of mismatch between pollinator size and flower morphology for some of the size guilds.

All pollinators were captured, identified and weighted in the lab. Based on their weight the bees were assigned to the following size classes (hereafter referred to as *size guilds*): small bees (including *Lasioglossum* spp.), small–medium bees (including *Megachile* spp., *Osmia* spp. and *Anthidium* spp.), medium–large bees (including *Andrena* spp. and *Eucera* spp.), and large bees (including *Anthophora* spp. and *Bombus* spp.).

Reproductive success was affected by the visitation frequency of the different bee size classes, with higher visitation rates of medium–large (*Andrenidae*) and large (*Apidae*) bees increasing seed set, while higher visitation rates of small (*Halictidae*) and small−medium (*Megachilidae*) bees reduced seed set (Vivarelli & al. 2011). The data above suggest that the influence of bee size on seed set is related to the different foraging techniques on flowers. In fact, the four size guilds varied in their handling time per flower. This variation was mainly due to the small bees having a significantly longer handling time per flower than the other guilds. Large bees let the pollen dispenser mechanism "recharge" after their short visits, removing only one "batch" of pollen, not promoting self pollination within the flower. Small bees on the other hand, were able to enter the flower and collect pollen while moving freely inside it, thereby activating the pump-like pollen dispensing mechanism of the flowers several times inducing a higher degree of selfing compared to larger bees. As the plant is self-incompatible, we may suppose that the reduced seed set after visitation by smaller bees are caused by stigma clogging with the flower's own pollen preventing successful application of co-specific, non-self pollen (see also Shore & Barrett 1984; Scribailo & Barrett 1994; Murphy 2000). Also, the analysis of pollen loads on the scopae and corbiculae of the four pollinator size guilds showed a lower fidelity for small–medium bees (*Megachilidae*) compared to the other three bee size guilds This could increase the probability of passing own pollen to stigmas within the same flower.

Discussion and Conclusions

As pollinator characteristics may influence floral traits over evolutionary time (Darwin 1862; Stebbins 1970), so plant characteristics may affect the foraging decision of animals in ecological time (Schemske & Horvitz 1984; Armbruster 1988).

As Fenster & al. (2004) pointed out, evolutionary specialization onto functional groups does not necessarily mean "ecological" specialization. The first has been defined by Olesen & Jordano (2002) as "the process of evolving in the direction of more specialization" whereas the latter refers to a state of a plant interacting with one to few insect species. Stebbins (1970) tried to solve the apparent paradox between evolutionary specialization and ecological generalization, regarding to both the quantity and quality components of animal activity as selective forces: "since selection is a quantitative process, the characteristic of a flower will be moulded by those pollinators that visit it most frequently and effectively in the region where it is evolving".

The evidence discussed above about the differential effect of size, structure, and ethology of pollinating and visiting insects on the plant fitness suggests that most legume species that appear to be generalist because of the large spectrum of visiting insects, are ecologically specialists, in that their fitness is conditioned by only one or few pollinating species. Such ecological specialization may account for homoplasy of floral characteristics, due to common pollination syndromes rather than to common ancestry. If so, the inconsistence between morphologically based "natural" taxa, and clades based on molecular phylogeny may be explained as the outcome of biologically different processes. So, we may well admit that several "natural" genera may not represent monophyletic clades. Of course, the discussion remains open, how far taxonomy should adhere to the one or the other evidence.

References

Armbruster, W. S., 1988: Multilevel Comparative Analysis of the Morphology, Function, and Evolution of *Dalechampia* Blossoms. – Ecology **69:** 1746-1761.

Cantó, P., Rivas-Martínez, S., Greinwald, R. & van Rensen, I. 1997: Revisión de *Genista* L. sect. *Spartioides* Spach en la Península Ibérica y Baleares. – Lazaroa **18:** 9-44.

Cristofolini, G. & Conte, L. 2002 : Phylogenetic patterns and endemism genesis in *Cytisus* Desf. (*Leguminosae* – *Cytiseae*) and related genera. – Israel J. Pl. Sci. **50:** S37-S50.

Darwin, C. 1862: On the various contrivances by which British and foreign orchids are fertilised by insects : and on the good effects of intercrossing. – London.

Faegri, K. & van der Pijl, L. 1971: The principles of pollination ecology. – Oxford & New York.

Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R., Thomson, J.D. 2004: Pollination syndromes and floral specialization. Annual Rev. Ecol. Evol. Syst. **35:** 375-403.

Free, J. B. 1993: *Insect Pollination of Crops*. – London.

Galloni, M. & Cristofolini, G. 2003 : Floral rewards and pollination in *Cytiseae* (*Fabaceae*). – Pl. Syst. Evol. **238:** 127-137.

—, Podda, L., Vivarelli, D., Cristofolini, G. 2007: Pollen presentation, pollen-ovule ratios, and other reproductive traits in Mediterranean Legumes (Fam. *Fabaceae* – Subfam. *Faboideae*). – Pl. Syst. Evol. **266:** 147-164.

—, —, —, Quaranta, M., Cristofolini, G. 2008 : Visitor diversity and pollinator specialization in Mediterranean legumes. – Flora **203(1):** 94-102.

Gibbs, P. E. 1966 : A revision of the genus *Genista* L. – Notes Roy. Bot. Garden Edinburgh **27(1):** 11-99.

— 1967 : A revision of the genus *Adenocarpus*. – Bol. Soc. Brot. **41:** 67-121.

— & Dingwall, I. 1971: A revision of the genus *Teline*. – Bol. Soc. Brot. **45:** 269-316.

Greuter, W., Burdet, H. M., Long, G. (eds.) 1989: Med-Checklist: A critical inventory of vascular plants of the circum-mediterranean countries, **4.** – Genève.

Herrera, C. M. 1987 : Components of pollinator "quality": comparative analysis of a diverse insect assemblage. – Oikos **50:** 70-90.

Johnson, S. D., Steiner, K.E. 2000: Generalization versus specialization in plant pollination systems. – Trends Ecol. Evol. **15:** 140-143.

Käss, E.& Wink M. 1995: Molecular Phylogeny relationships of the *Papilionoideae* (family *Leguminosae*): RbcL Gene Sequences versus Chemical Taxonomy. – Bot. Acta **108:** 149-162.

— & — 1997: Phylogenetic relationships in the *Papilionoideae* (family *Leguminosae*) based on nucleotide sequences of cpDNA *(rbc*L*)* and ncDNA (ITS1 and 2). – Mol. Phyl. Evol. **8:** 65-88.

Kay, K. M. & Schemske, D. W. 2004: Geographic patterns in plant-pollinator mutualistic networks. – Ecology **85:** 875-878.
Larsson, M. 2005: Higher pollinator effectiveness by specialist than generalist flower-visitors of unspecialized *Knautia arvensis* (*Dipsacaceae*). – Oecologia **146:** 394-403.
Moeller, D. A. 2005: Pollinator community structure and sources of spatial variation in plant-pollinator interactions in *Clarkia xantiana* ssp *xantiana*. – Oecologia **142:** 28-37.
Murphy, S.D. 2000: Field testing for pollen allelopathy: a review. – J. Chem. Ecol. **26:** 2155-2172.
Olesen, J.M. & Jordano, P. 2002: Geographic patterns in plant-pollinator mutualistic networks. – Ecology **83:** 2416-2424.
Ollerton, J. 1996: Reconciling ecological processes with phylogenetic patterns: the apparent paradox of plant-pollinator systems. – J. Ecol. **84:** 767-769.
Olsen, K. M. 1997: Pollination effectiveness and pollinator importance in a population of *Heterotheca subaxillaris* (Asteraceae). Oecologia **109:** 114-121.
Pardo, C., Cubas, P., Tahiri, H. 2004: Molecular phylogeny and systematics of *Genista* (*Leguminosae*) and related genera based on nucleotide sequences of nrDNA (ITS region) and cpDNA (*trn*L-*trn*F intergenic spacer). – Pl. Syst. Evol. **255:** 93-119.
Pérez-Bañon, C., Juan, A., Petanidou, T., Marcos-García, M. A., Crespo, M. B. 2003: The reproductive ecology of *Medicago citrina* (Font Quer) Greuter (*Leguminosae*): a bee-pollinated plant in Mediterranean islands where bees are absent. – Pl. Syst. Evol. **241:** 29-46.
Petanidou, T., Lambron, E. 2005: A land for flowers and bees: studying pollination ecology in Mediterranean communities. – Pl. Biosyst. **139:** 279-294.
—, Potts, S. 2006: Mutual use of resources in Mediterranean plant-pollinator communities: how specialized are pollination webs? In: Waser, N.M., Ollerton, J. (eds.): *Plant-pollinator interactions: from specialization to generalization*. – Chicago & London.
Polhill, R. M. 1976: *Genisteae* (Adans.) Benth. and related tribes. – Bot. Syst. **1:** 143-368.
— & Raven, P. H. (eds.) 1981: Advances in Legume Systematics, **1-2.** – Kew.
Rodriguez-Riaño, T., Ortega-Olivencia, A., Devesa, J. A. 1999: Reproductive biology in two *Genisteae* (*Papilionideae*) endemic of the western Mediterranean region: *Cytisus striatus* and *Retama sphaerocarpa*. – Canad. J. Bot. **77:** 809-820.
Schemske, D. W. & Horvitz, C. C. 1984: Variation among Floral Visitors in Pollination Ability: A Precondition for Mutualism Specialization. – Science **225:** 519-521.
Schlindwein, C. & Medeiros, P. C. R. 2006 : Pollination in *Turnera subulata* (*Turneraceae*): Unilateral reproductive dependence of the narrowly oligolectic bee *Protomeliturga turnerae* (*Hymenoptera, Andrenidae*). – Flora **201:** 178-188.
Scribailo, R. W. & Barrett, S. C. H. 1994: Effects of prior self-pollination on outcrossed seed set in tristylous *Pontederia sagittata* (*Pontederiaceae*). – Sexual Pl. Reprod. **7:** 273-281.
Shore, J. S. & Barrett, S. C. H. 1984: The effect of pollination intensity and incompatible pollen on seed set in *Turnera ulmifolia* (*Turneraceae*). – Canad. J. Bot. **62:** 1298-1303.
Stebbins, G. L. 1970: Adaptive Radiation of Reproductive Characteristics in Angiosperms, I: Pollination Mechanisms. – Ann. Rev. Ecol. Syst. **1:** 307-326.
Talavera, S., Aedo, C., Castroviejo, S., Romero Zarco, C., Sáez, L., Salgueiro, F. J., Velayos, M. 1999: *Cytiseae* . – Pp. 44-260 in: Flora Iberica, **7(1).** – Madrid.
— & Salgueiro, F. S. 1999: Sobre el tratamiento de la tribu *Cytiseae* Bercht. & J. Presl. (*Papilionoideae, Leguminosae*) en "Flora Iberica". – Anal. Jard. Bot. Madrid **57(1):** 200-218.
Tepedino, V. J., Sipes, S. D., Griswold, T. L. 1999 : The reproductive biology and effective pollinators of the endangered beardtongue *Penstemon penlandii* (*Scrophulariaceae*). – Pl. Syst. Evol. **219:** 39-54.

Vivarelli, D., Petanidou, Th., Nielsen, A., Cristofolini, G. 2011: Small-size bees reduce male fitness of the flowers of *Ononis masquillierii* (*Fabaceae*), a rare endemic plant in the northern Apennines. – Bot. J. Linn. Soc. **165:** 267-277.

Waser, N. M. & Ollerton J. (eds.) 2006: Plant Pollinator Interactions – from specialization to generalization. – Chicago & London.

—, Chittka, L., Price, M.V., Williams, N. M., Ollerton, J. 1996: Generalization in pollination systems, and why it matters. – Ecology **77:** 1043-1060.

Woitke, M., Wolf, R., Hartung, W., Heilmeier, H. 2006: Flower morphology of the resurrection plant *Chamaegigas intrepidus* Dinter and some of its potential pollinators. – Flora **201:** 281-286.

Yeo, P. 1993: Secondary pollen presentation: form, function and evolution. – Pl. Syst. Evol., **Suppl. 6:** 1-268.

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