

Short communication

A phylogenetic exercise for the study of plants-herbivores interactions: the genus *Piper* as a case study

Abstract

Phylogenetic is a tool for evolutionary ecology useful to interpret the species properties and recently biological interactions based on historical rigorous hypotheses. Some of the most important ecological and evolutionary components in the plant-herbivore interface is the plant's defense pattern. In this study, we develop an exercise where we might give inferences of the evolutionary histories of a plant and its phytophagous using as a model to several species of *Piper* genus. With bibliographic data, we tested the effect of a number of herbivores, disturbance, pubescent and soil type in 11 species of *Piper* and their historical relationships with anti-herbivore characteristics. The pubescent and disturbance was the most important characteristics, can assume that pubescence is not associated with herbivory traits, but probably have an adaptative value. We conclude that the uses of a historical approach to explaining ecological phenomena have several applications, especially in areas that are usually based on adaptive planning, for the explanation of phenomena, and we show a simple and useful procedure to make evolutionary inferences and to generate practical cases for the teaching of evolutionary ecology.

Introduction

The phylogenetic focus is a tool for evolutionary ecology that has developed a conceptual framework to interpret the properties and distributions of species, based on evolutionary rigorous hypotheses (Simpson and Cracraft 1995). Knowledge the phylogenetic history is proving to be critical in understanding community interactions. Nowadays this type of approaches has been used to study biological interactions, to developing a hypothesis about the processes of evolutionary change between interacting species and to propose evolutionary associations between the species and their characteristics that present narrow links and indicate coevolutionary or macroevolutionary processes (Mitter and Farrell 1992). In general, the ecology of plant-animal interactions is widely acknowledged, nevertheless is very limited the knowledge about long-term evolutionary patterns and the processes by which interactions have been generated (Armbruster 1997). In this respect, the line to be explored would be to identify the processes and historical conditions that have developed changes in the plant-herbivore interface.

Some of the most important ecological and evolutionary components in the plant-herbivore interface is the plant's defense patterns and syndromes (Agrawal, 2007). These influence the dynamics of the populations, the distribution of plants and herbivores. Therefore, the variation between species or communities of anti-herbivore mechanisms is a reflection of the diverse forms, styles, and patterns in which animals and plants can interact. Therefore, to consider how the diverse defensive patterns develop on the plants from a historical point of view may provide interesting information for the understanding of the plant-herbivore interface (Richards et al 2010).

In this study, we develop an exercise where we might give inferences of the evolutionary histories among species plant and its phytophagous insects associated using as a model to Piper genus. For this, our evolutionary hypotheses are the following; we expected that closer related species exhibit similar characteristics to each other; that the presence and insistence of pubescence on seedlings is a character with anti-herbivore influence which is the product of nearby evolutionary histories, and that plants that grow in environments with specific light and nutrient interactions will have the ability to deploy similar anti-herbivore strategies for a historical effect.

Methods

For this interpretation exercise, we select a phylogeny based on DNA ribosomal (Jaramillo and Hands, 2001) and associate each neotropical Piper species with the list of herbivore preference characteristics presented by Marquis (1992). The characteristics that Marquis indicates to describe the patterns of herbivorous in Piper are: a) number of herbivores, which indicates the susceptibility of each taxa of being attacked; b) disturbance, indirectly indicates the level of light resources to which plants are exposed in primary forest (F), characterized as shade tolerant with little access to carbon (route light) and with potential to express chemical defenses based nitrogen. Plants associated with secondary environments (S) are typically classified as pioneers with high growth rates and low investment in defenses; c) pubescence, generally associated with an anti-herbivore defense, preventing oviposition and access from the insect's jaws to plant structures; and d) Soil type, its refers to the access of plants to nutrients, in general, the theory indicates that plants with poor access to nutrients can deploy more intense anti-herbivorous strategies than plants that grow in rich environments (Coley 1985, Stamp 2003). This variable

may be related to disturbance and establish a carbon-nitrogen ratio. Once realized the contrast of the information, our approach is to determine if there is a historical influence on the display of anti-herbivorous characteristics of the different rates. In order to corroborate the hypothesis of the relationship between pubescence and type of environment and the number of herbivores - and thus assign this character, an anti-herbivore value - develop a Mann-Whitney test (Zar 2013).

Results

The variables of maximum size and abundance were not considered because of the low precision with which they are raised in the work of Marquis. The ecological attributes, on the defense mechanisms for each of the species, were the following: 1) *Piper Darienense*: It predicts a shadow tolerance strategy (low growth) with high accumulation of secondary metabolites. No presence of pubescence or other mechanical defenses are reported; 2) *Piper reticulatum*: Due to it develops in a rich environmental, it will deploy a strategy of growth without investment defenses. No pubescence is reported; 3) *Piper garagaranum*: It develops in closed forest (tolerant), high chemical defenses investment is expected, presented in addition, high levels of pubescence; 4) *Piper arieianum*: It develops in closed forest, in all soil types, with little pubescence presence, chemical defenses are expected; 5) *Piper augustum*: Due to the substratum, chemical defenses are expected. Present in all soils. Little pubescence; 6) *Piper multiplinervium*: It occurs in all types of soils, lots of light, high growth. It is expected deployment growth at the expense of investment in defense. Without pubescence; 7) *Piper auritum*: Occurs in all soils, with lots of light, high growth. It is expected growth in deployments at cost of investment in defense of herbivory. This species presents little pubescence; 8) *Piper arborium*: It is present in all soils, few pubescence, high number of herbivory attacking it. Chemical strategies are expected; 9) *Piper imperiale*: It occurs in all soils, few pubescence, low mechanical defenses, is attacked by a large number of herbivores, therefore chemical strategies are expected; 10) *Piper hispidum*, occurs in all soils, open terrain, high growth. Little defense with growth display expected; and 11) *Piper aduncum*, occurs in all soils, open terrain, high growth defense.

Due to that was not counting for all the species of environmental characteristics, we filter the proposed tree respecting its hierarchy within the cladogram (Fig 1). The proposed tree analysis indicates that the *Piper darienense* clade consisting of *Piper reticulatum* and represents

the baseline group of taxa tested (Fig. 1). Due to the lack of consistency in the ecological data center the analysis on two components: pubescent and disturbance.

a) *Pubescence*

It argues that pubescence may be a derivative character as it is not presented in the most ancestral clades (*Piper Darienense* and *Piper reticulatum*). In the clade 2 (*P. gaganum*) observed this character emerges in the most basal taxa, whereas *P. aireanum* and *P. augustum* the character does not arise. However, these two taxa have a high level of herbivores attack, in this context and a historical point of view is likely to be a derived pubescence character associated with herbivorous for this clade. In contrast, in the clade 3 (*P. multiplinervium*), some taxa "win" pubescence while those not present response to an ancestral effect (*P. multiplinervium* and *P. arboreum*).

The contrast between pubescence and the number of herbivores - did not show significant differences ($U = 11$; $P < 0.83172$), that is to say, the rates with hairs are equally attacked than the hairless rates. This result can assume that pubescence is not associated with herbivory feature, but probably if you have an adaptive value (like ecophysiological trait), which makes them appear in different parts of cladograms and this defensive partnership occurs at certain clades product of differential selective pressures.

b) *Disturbance*

To develop in environments secondary vegetation (S) or primary forest (F) is a feature which could not be resolved if the character state is ancestral (F or S). However at the apical levels of the topology, character states are monophyletic, that is, we observe that neighboring taxa share environments. This leads us to infer that at this level (groups of species) there may be a historical effect in as for the exploitation of niches.

With regard to anti-herbivore associated with the ambient interference and, we predict that the apical groups that share similar environments should deploy defensive strategies. However, due to limited data on herbivory rates or quantifications on structural, or other chemical defenses (Richards et.al. 2010, Myers et al 2008), it is not possible to reinforce this historical assertion.

Theoretically, plants should be in closed forests and should adapt strategies of resistance to herbivores (by chemical defenses), while plants in areas of secondary vegetation would show

little defense (Coley et al., 1985). However, with the data provided by Marquis, no relationship was found between numbers of herbivores and the type of environment where the plant is developed ($U = 10.5$; $P < 0.74912$). These predictions can be altered when we change the scale of the study, that is, to evaluate the predictions at the population level (which should be similar according to the environment in which they are developed), or by the other to include in the analysis higher levels as **genders**. This approach could refine the analyzes and give us clearer evolutionary histories that explain the patterns of defense in the plant.

Discussion

To properly explore an ecological phenomenon from a historical point of view (in this case, the relationship the deployment of defensive strategies of the genus *Piper*), it would be important to have more precise about their levels of damage of different species, also an analysis of chemical (S da Silva, et al., 2016) and mechanical defense compounds like **pubescence**, not forgetting alternative strategies not always considered as the induced responses or traits (Uesugi, et al. 2017). It would also be necessary to increase the number of taxa represented in clades with emphasis on species of neotropical origin.

On the other hand a finer analysis should consider the phylogeny of herbivores associated with *gender Piper*, this could give us basis to explore whether there is a **correspondence** to evolving between clades (Rasman and Agrawal 2009), and to investigate if the cladogenesis of both groups has been measured by the appearance and disappearance of defenses and counter-fights in interaction (Richards et al. 2010), this would give us clear evidence of potential "arms wars" between herbivores and plants.

Another scenario worthy of being evaluated by phylogenetic systematics **is** the defense systems associated with the architecture of plants (Forber et al., 2017). In plant-herbivore **theory**, it has been considered very little that aspects such as fragmentation of the leaf area may be a character that makes them less susceptible to plants being attacked by herbivores. In this **direction**, a serious hypothesis is that plants with fragmented foliar structures should have lower levels of damage, due to the decrease of the area of "attack" of the herbivore. With this scenario it would be interesting to be able to make a historical analysis of the groups of plants that present this fragmentation of the area (e.g. ferns, legumes, etc.) and associate them with levels of damage between species and if possible contrast it with the phylogenies of the groups of herbivores that

attack these plants (see Futuyma and Agrawal, 2009). It is likely that the characteristics of fragmenting leaves in plants appear independently in several clades, this character associated with low levels of herbivory to it could give us an indication of being an alternative system of defense, however since the fragmentation of the leaves may have alternative physiological explanations (e.g. rupture of the boundary layer) it would be appropriate to make a balance of the cost-benefit of preserving this character.

Conclusion

As mentioned, the uses of a historical approach to explaining ecological phenomena have varied applications, especially in areas that are usually based on adaptive planning, for the explanation of phenomena, as does most of the theory that is around plant-herbivore interactions. The procedure proposed here can be used as a preliminary exploration tool and used in groups where the phylogeny is known (Webb Et al. 2008) and data can be taken that modulate the interactions (e.j. herbivory, pollination).

We recommend the use of this methodology to make a historical approach that allows linking ecological characters like defense syndrome with damage levels of plants. One possibility is to use global databases such as Phylocom (Webb Et al. 2008), which can be very useful especially in the teaching of evolutionary ecology.

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