1   **Testing host-plant driven speciation in phytophagous insects: a phylogenetic perspective**

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11

12   **Abstract**

13   During the last two decades, ecological speciation has been a major research theme in

14   evolutionary biology. Ecological speciation occurs when reproductive isolation between

15   populations evolves as a result of niche differentiation. Phytophagous insects represent model

16   systems for the study of this evolutionary process. The host-plants on which these insects feed and

17   often spend parts of their life cycle constitute ideal agents of divergent selection for these

18   organisms. Adaptation to feeding on different host-plant species can potentially lead to ecological

19   specialization of populations and subsequent speciation. This process is thought to have given

20   birth to the astonishing diversity of phytophagous insects and is often put forward in

21   macroevolutionary scenarios of insect diversification. Consequently, numerous phylogenetic

22   studies on phytophagous insects have aimed at testing whether speciation driven by host-plant

23   adaptation is the main pathway for the diversification of the groups under investigation. The

24   increasing availability of comprehensive and well-resolved phylogenies and the recent

25   developments in phylogenetic comparative methods are offering an unprecedented opportunity to

26   test hypotheses on insect diversification at a macroevolutionary scale, in a robust phylogenetic

27   framework. Our purpose here is to review the contribution of phylogenetic analyses to investigate

28   the importance of plant-mediated speciation in the diversification of phytophagous insects and to

29   present suggestions for future developments in this field.

Introduction

The idea according to which new species arise through adaptation to different ecological niches constitutes the core of Darwin's work. This process is now termed ecological speciation and its study has become an intense field of research in evolutionary biology (Nosil, Crespi, Sandoval 2002; Rundle, Nosil 2005; Schluter 2009; Nosil 2012). **Phytophagous insects***[1] have always been at the forefront of these investigations (Drès, Mallet 2002; Funk, Filchak, Feder 2002; Matsubayashi, Ohshima, Nosil 2010; Elias et al. 2012; Forbes et al. 2017). The hypothesis of ecological speciation resulting from divergent selection exerted by host-plants was put forward a long time ago to explain the formation of new species of insects (Brues 1924). There are several model systems on which this scenario has been explored. One text-book example of host-plant driven incipient speciation is the apple maggot (*Rhagoletis pomonella* complex) in which the evolution of new feeding preferences on the recently introduced domesticated apple (*Malus pumila*) has supposedly led to the emergence of specialized host races (Bush 1975; Berlocher 2000; Powell et al. 2014). Stick insects, leaf beetles (Nosil et al. 2012; Rundle et al. 2000), butterflies (McBride, Singer 2010), and the pea aphid also star among model systems in the study of host-driven speciation (Via, Bouck, Skillman 2000; Caillaud, Via 2000; Peccoud et al. 2009; Smadja et al. 2012). In all these examples, the speciation scenario hypothesizes that: 1) the restricted utilization of distinct sets of host-plant species by insect populations is the result of adaptive trade-offs; 2) hybrids with intermediate phenotypes (in terms of traits involved in **host-plant adaptation***) fare poorly on parental host-plants and are selected against and therefore gene flow between populations is reduced; 3) gene flow can further be reduced through the evolution of assortative mating, especially when host-plants also represent mating sites. In support of this scenario, many studies show the existence of genetically differentiated host races in insect species. Some studies have quantified selection against hybrids (McBride, Singer 2010; Gow, Peichel, Taylor 2007) and some studies have uncovered genomic regions that determine host-plant preference and performance on alternative hosts (Egan, Nosil, Funk 2008; Smadja et al. 2012).

The role of host-plant-mediated speciation in the diversification of phytophagous insect lineages is also largely emphasized in the literature on large-scale patterns of insect diversity: macroevolutionary perspectives on phytophagous insect evolution have attributed their extraordinary diversification to selective responses to their host-plants (Ehrlich, Raven 1964; Yokoyama 1995; Winkler, Mitter 2008; Janz 2011). However, these macroevolutionary scenarios are often presented in the literature as narratives for specific lineages commenting a phylogenetic

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[1] See glossary

62   reconstruction of the history of host-plant associations. Many phylogenetic studies still fail to

63   clearly formulate hypotheses and predictions about the speciation processes that underlie the

64   observed patterns and the role played by host-plant adaptation in those. The reason might be that

65   the macroevolutionary patterns that arise when **host-plant specialization*** is the driver of

66   speciation events are not always clear. There is no review on what to expect and how to formally

67   test these predictions.

68   The increasing availability of robust molecular phylogenies and recent developments in

69   phylogenetic comparative methods are offering an unprecedented opportunity to test evolutionary

70   hypotheses in a robust phylogenetic framework. Our purpose here is to present the

71   macroevolutionary scenarios for the diversification of phytophagous insects that have been put

72   forward in the literature, decipher the role that ecological speciation driven by host-plant

73   adaptation play in them and synthetize predictions from these scenarios. We then identify tools

74   from the "comparative phylogenetic toolbox" that provide ways to test some of these predictions.

75   This toolbox can be divided into three compartments:

76   1)   comparisons of the phylogenetic histories of insects and their associated plants: the

77   congruence (in terms of dates of divergence and branching patterns) of the phylogenetic histories

78   of plant-feeding insects and their host-plants can be tested in robust statistical frameworks and

79   illuminate how herbivores track the diversification of their hosts;

80   2)   ancestral character state reconstructions: the evolutionary trajectory of host-associations,

81   host breadth and host-plant adapted traits can be inferred using ancestral character state

82   reconstruction methods and statistical tests can determine whether their distribution throughout the

83   phylogenetic trees follow the predictions of scenarios involving ecological speciation mediated by

84   host-plant adaptation;

85   3)   diversification analyses: the recent developments of methods to study the **diversification**

86   **dynamics*** of entire clades using phylogenetic trees provide ways to test how shifts to new host-

87   plant species or changes in host breadth have impacted diversification rates in phytophagous

88   insects.

89   We review papers that have adopted these approaches. We then present suggestions for future

90   research that should help linking microevolutionary studies on host-plant adaptation and

91   macroevolutionary perspectives on phytophagous insect diversification.

92   **I Macroevolutionary scenarios of phytophagous insect diversification**

93   **I.1)** *Escape and radiate* (**Figure 1 a**)

More than 50 years ago, Ehrlich & Raven (Ehrlich, Raven 1964) put forward a macroevolutionary scenario that inspired most of the current research on plant-feeding insect diversification: it is known as "***Escape and radiate***" (Thompson 1989). They hypothesized that when insects acquire the ability to circumvent the chemical defenses of a plant group, it promotes their rapid diversification by ecological release, *i.e.* the availability of novel resources and reduction in direct competition. Insects undergo an **adaptive radiation***. In this scenario, adaptation towards host-plants is the driving force of insect species formation. The "*Escape and radiate*" scenario also hypothesizes that, in response to phytophagous insect predation, plants acquire novel chemical defenses which allow them in turn to diversify very rapidly (Marquis et al. 2016). Ehrlich & Raven' s seminal study suffers from several shortcomings that have been pinpointed before (Janz 2011). First, although the authors frame their theory within the concept of adaptive radiation, they do not explicitly lay out some speciation mechanisms for both partners of the interaction. Following their scenario, a trade-off in resource use and specialization towards specific host-plants is necessary to explain the formation of numerous insect species (*i.e.,* species radiation) following the capture of a new host-plant lineage. Such a trade-off is not mentioned in the original paper (Janz 2011). In addition, as underlined by contemporary researchers of Ehrlich and Raven's, it is difficult to conceive how the selection pressures exerted by insects on plant defences can drive plant speciation (Jermy 1976; Jermy 1984). Plant traits that reduce phytophagous insect attacks are rarely linked with reproductive isolation between plant populations (but see Marquis et al. 2016) for a review of scenarios of herbivore-induced speciation in plants) and the evidence for bursts of speciation in plants following the evolution of chemical defence is scant (Futuyma, Agrawal 2009). However this study has been and remains a great source of inspiration for studies on the diversification of plant-insect associations. This is probably because it is one of the first studies that attempts to explain how microevolutionary processes (host-plant adaptation) translate into macroevolutionary patterns (radiation onto newly acquired plant lineages). Several predictions that can be tested on phylogenetic trees have been derived from the *Escape & Radiate* scenario (Table 1).

In the years following its publication, "*Escape and radiate*" was often interpreted as generating **cospeciation*** patterns; however it is now recognized that it rather predicts the sequential speciation of insects onto an already diversified plant lineage (Janz 2011; Suchan, Alvarez 2015). According to this prediction: 1) the reconstruction of the history of host-plant associations on the phylogenetic trees of insects should reveal **host-plant conservatism***, *i.e.* the use of related plant species by related insects (Winkler, Mitter 2008); 2) the phylogenies of herbivorous insects and their host-plants should be more congruent than expected by chance and
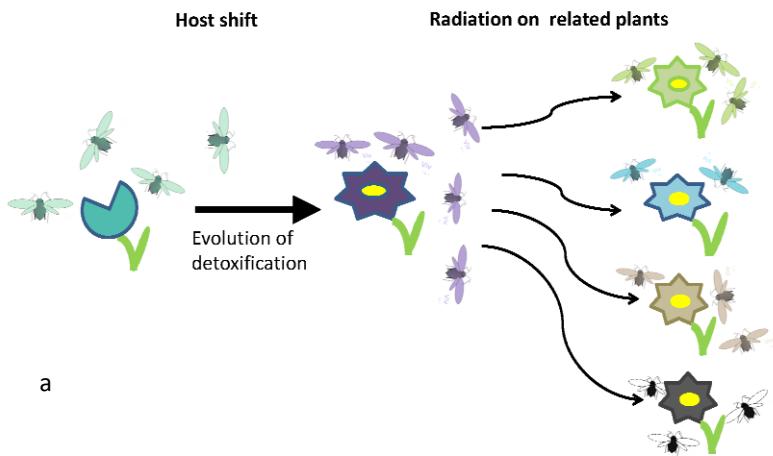
128   the diversification of the insects should lag behind that of their host-plants; this is called sequential
129   evolution (Jermy 1984) or "*host tracking*". Nevertheless, when the association between insects
130   and their host plants is species-specific, a pattern of cospeciation can be expected through simple
131   co-vicariance: geographic barriers affect the differentiation of populations of interacting lineages
132   in a similar way and cause simultaneous speciation events (Althoff et al. 2012; Brookes et al.
133   2015; Martínez-Aquino 2016). In these cases, it is geographic isolation and not natural selection
134   that initiates the reproductive isolation of insect populations and subsequent speciation. However,
135   the specificity of the insects and host-adapted traits enhance the probability of shared vicariant
136   events.

137   The diversification dynamics of insects should follow the typical pattern of adaptive
138   radiations (Janz 2011), *i.e.* they should show an acceleration of speciation rate upon the capture of
139   new plant lineages or the evolution of detoxification mechanisms (Wheat et al. 2007) and then
140   slow down when their niches are saturated (when species diversity is reaching the carrying
141   capacity of the host-plant lineage) (Rabosky, Lovette 2008). Furthermore, the capture of a species-
142   rich clade of plants should result in higher speciation rates than the capture of lineages
143   encompassing less species (Roskam 1985).

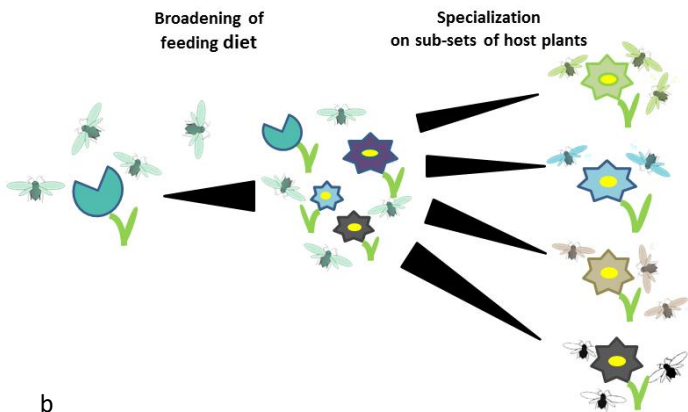### I.2) The *'Oscillation Hypothesis'* (Figure 1b)

145   The "*Escape and radiate*" scenario was revisited more than a decade ago by Janz and
146   collaborators (Janz, Nylin, Wahlberg 2006; Janz, Nylin 2008; Nylin, Janz 2009). Using butterflies
147   as study systems, they stated that expansions in diet breadth followed by specialisation onto new
148   host-plant species constantly fuel the diversification of phytophagous insects. This has been
149   termed the '***Oscillation Hypothesis***' (Janz & Nylin 2008). It stipulates that transitions towards a
150   generalist diet generally open up a new adaptive zone, which favours the capture of new host-
151   plants. In this scenario, expansions in diet are enabled by the phenotypic plasticity of insects with
152   respect to host-plants (Nylin, Janz 2009). Population fragmentation and their specialisation onto
153   newly captured host-plants then lead to the formation of new species. Hence this scenario
154   explicitly predicts that species formation results from insect populations evolving towards the
155   utilization of a restricted set of host-plants. Nevertheless, it suggests that this specialization
156   process is often a consequence of the geographic isolation of generalist insect populations in areas
157   inhabited by different host-plant species (Janz, Nylin, Wahlberg 2006). Therefore the "*Oscillation
158   hypothesis*" does not necessarily postulate that natural selection is the main driving force of
159   species formation.
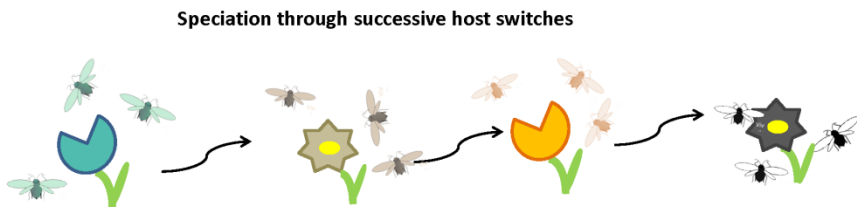
Escape and Radiate (Erlich & Raven 1964)

Host shift   **Radiation on related plants**

a

160

Oscillation hypothesis (Janz & Nylin 2008)

Broadening of   **Specialization**
feeding diet   **on sub-sets of host plants**

b

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Musical chairs (Hardy & Otto 2014)

Speciation through successive host switches

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c

163

164   Figure 1: Schematic illustrations of the macroevolutionary scenarios of phytophagous insect
165   diversification.

166   However, subsequent papers quoting this scenario emphasize the central role of host-plant
167   specialisation (Hardy, Otto 2014; Nakadai 2017; Wang et al. 2017).

168   Most of the predictions from the *Escape and Radiate* scenario are valid under the
169   *Oscillation* hypothesis. However, the latter hypothesis yields several new predictions (Table 1):

170   - **generalist*** diets should be "transient and repeatedly disappear in favour of specialization
171   onto a limited set of related plants" (Nylin, Slove, Janz 2014);

172   - gains of new host plants are associated with host breadth expansion (Janz *et al.* 2001);

173   - the amplitude of the oscillation determines the number of potential host-plant species,
174   therefore, insect clades with the most diverse host-use (the highest number of host-plant species)
175   are expected to be more speciose than clades using fewer host species (Janz, Nylin, Wahlberg
176   2006) and insect clades that encompass species that exhibit large host breadths should have higher
177   speciation rates (Weingartner, Wahlberg, Nylin 2006; Hardy, Otto 2014);

178   - shifts from a generalist diet to a specialist one should be associated with an acceleration
179   of speciation rates. In other words, patterns of diversification should follow a model where
180   cladogenetic events are associated with host breadth reduction (Hardy, Otto 2014);

181   - generalists have larger geographic ranges as they are able to colonize more habitats and
182   can expand more easily (Slove, Janz 2011).

183   **I.3) The *Musical Chairs* (Figure 1c)**

184   As opposed to the *Oscillation* hypothesis, Hardy and Otto (2014) proposed an alternative
185   scenario in which speciation in herbivorous insects is driven by host-switching rather than
186   transitions in diet breadth. The authors named their scenario *"**the Musical Chairs**"* (Hardy, Otto
187   2014). In this scenario, phytophagous insects speciate by the sequential capture of new host-plants
188   and therefore the speciation of a plant-feeding insect lineage is merely driven by the number of
189   plants it can colonize. Host-driven speciation remains at the centre of this hypothesis.

190   The "*musical chairs*" hypothesis yields several predictions that differentiate it from the
191   previous macroevolutionary scenarios (Table 1):

192   - contrary to the *Escape* and *Radiate* hypothesis, the capture of a new host plant lineage
193   does not initiate a radiation;

194   - contrary to the oscillation hypothesis, gains of new hosts are not associated with host
195   breath expansion (Hardy 2017), host breadth contraction is not associated with cladogenetic events
196   (Hardy, Otto 2014) and overall there should be a negative correlation between host-plant breadth
197   and speciation rates (Hardy, Otto 2014);

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198   - speciation rates are higher in insect lineages that exhibit **lability*** in host-plant
199   associations.

200   The lack of connection between host breath contraction and speciation events and the
201 negative association between host-breadth and speciation rates clearly differentiate the *Musical*
202 *chairs* from the *Oscillation hypothesis*. However, several authors have pointed out that if
203 generalist feeding diets are indeed ephemeral as expected when specialization towards host-plants
204 is adaptive, it will be difficult to reconstruct its history accurately on phylogenetic trees (Hardy,
205 Peterson, Normark 2016; Janz et al. 2016). Consequently, the relationships between host breath
206 and speciation rates will be difficult to explore and the set of predictions that differentiate the
207 *Musical chairs* from the *Oscillation hypothesis* will not always be testable.

208   Phylogenetic comparative methods (Pennell, Harmon 2013) including increasingly
209 sophisticated diversification models (Rabosky 2006; Stadler 2013; Stadler, Bokma 2013;
210 Rabosky, Goldberg 2015; Beaulieu, O'Meara 2016; O'Meara, Beaulieu 2016) can now be
211 deployed to reconstruct ancestral character states, investigate the diversification dynamics of
212 insect lineages and test whether shifts in diversification rates are associated with transitions in
213 character states. Below we review how these methods have been used to investigate the evolution
214 of plant/insect associations and test the predictions of host-driven speciation scenarios.

II Phylogenetic approaches for testing ecological speciation scenarios

II.1) Comparing the phylogenies of plants and insects

217   Several phylogenetic studies have compared the phylogenies of herbivorous insect and
218 their host-plants. Some have investigated cospeciation using dedicated tools such as tree
219 reconciliation analyses (Page 1994; Conow et al. 2010) and distance-based methods for tree
220 comparisons (Legendre, Desdevises, Bazin 2002). These tools statistically test the null hypothesis
221 that the phylogenies of insects and their associated plants are more congruent than expected by
222 chance and that speciation events are simultaneous. As stated above, this is only expected in
223 species specific plant/insect interactions. As predicted by early taxonomic studies (Ramirez 1974;
224 Wiebes 1979), cospeciation has been evidenced on figs and their phytophagous pollinating wasps
225 but the degree of phylogenetic congruency observed varies according to taxonomic groups (see
226 review by de Vienne et al. 2013 on cospeciation studies and Cruaud et al. 2012). Reciprocal
227 adaptations of plants and insects (*i.e,* **coevolution***) have been unravelled in this study system
228 (Jousselin, Rasplus, Kjellberg 2003; Weiblen 2004). However, it is not known whether the
229 cospeciation patterns observed are the sole result of this coevolution, i.e. whether reciprocal

230   selection exerted by both partners has driven the reproductive isolation of interacting populations;
231   (Althoff, Segraves, Johnson 2014; Hembry, Yoder, Goodman 2014) or whether matching
232   speciation events have arisen through co-vicariance. In the other iconic model system for the study
233   of plant/insect **coevolutionary diversification***, the *Yucca*–Yucca moth interaction (Pellmyr
234   2003), moth phylogenies parallel some parts of the evolutionary history of their hosts. Some
235   studies suggest that this pattern is the result of coevolution (Godsoe et al. 2009) while others hint
236   towards co-vicariance (Althoff et al. 2012). In both *Yucca*-moths and fig wasps the fact that the
237   phytophagous insects are specific pollinators of their host-plants and breed inside them necessarily
238   links the reproductive success of the two partners and increases the likelihood of concomitant
239   speciation events. Therefore host-plant adapted traits are certainly pivotal in the speciation process
240   of these insects.

241   Studies that have investigated **phylogenetic tracking*** between phytophagous insects and
242   their host-plants have shown that insects rarely mimick their host-plant phylogenies (see reviews
243   by Winkler, Mitter 2008; de Vienne et al. 2013); a third of the 20 studies reported in these two
244   reviews found partial patterns of congruence between insects and host plant phylogenies. In many
245   plant/insect interactions, a simple observation of the patterns of distribution of host plants in the
246   insect phylogeny can actually rule out cospeciation or phylogenetic tracking and studies on plant-
247   insect interactions have moved away from cospeciation studies.

248   In order to test for successive burst of diversification in plants and insects, many authors
249   have thus simply compared the timing of divergence of plants and associated insects (*e. g*.,
250   (Gómez-Zurita et al. 2007; Leppanen et al. 2012; Brandle et al. 2005; Lopez-Vaamonde et al.
251   2006; Pena, Wahlberg 2008; McKenna et al. 2009; Stone et al. 2009; Kergoat et al. 2011; Segar et
252   al. 2012; McLeish, Miller, Mound 2013; Wahlberg, Wheat, Pena 2013; Kergoat et al. 2015; Vea,
253   Grimaldi 2016). Most of these studies suggest delayed colonization of already diversified groups
254   of plants by insects groups at different temporal scales. They are generally based on mere
255   qualitative comparisons of dates of divergence obtained from fossil calibrated phylogenies of both
256   plants and insects, but can also include thorough statistical comparisons of dates obtained through
257   phylogenetic methods (Loss-Oliveira, Aguiar, Schrago 2012; McLeish, Miller, Mound 2013).
258   They are often framed as supporting the *Escape and radiate* theory. However these studies do not
259   give any information on the speciation process behind the diversification of the insect lineages
260   studied, they merely indicate the timing of host plant colonization.

261   Table 1: Predictions from host-plant driven speciation scenarios: the first column indicates the evolutionary hypotheses tested and the headers
262   of the other columns indicate phylogenetic comparative approaches used for testing them.
263

Macroevolutionary scenarios	Insect and host tree comparison	Evolution of host associations	Evolution of host breadth	Insect diversification dynamics
Speciation driven by host-plant specialization	Cospeciation if the insects play a role in their host-plant reproductive isolation	No overlap in host use among sister-species (Nyman *et al.* 2010)	Predominance of specialists over generalists (Janz *et al.* 2001, Winkler & Mitter 2008)	- herbivorous insects are more diverse than their non-herbivorous relatives (Mitter *et al.* 1988) - the diversification dynamic of phytophagous insects follows a pattern of adaptive radiation (Janz 2011) - the number of species within an insect clade positively correlates with the number of host-plant species (Janz 2006)
Escape and radiate	Phylogenetic tracking (Jermy 1976, Mitter & Brooks 1983)	Phylogenetic conservatism of host-plant lineages or host-plants with similar defences (Winkler & Mitter 2008)	No prediction	Increase in speciation rates upon the capture of new plant lineages or detoxification mechanisms (Wheat *et al.* 2007: Fordyce 2010)
Oscillation	Potentially phylogenetic tracking following the capture of a new host-plant lineage	Conservatism of host-plants following the capture of a new plant lineage.	- lability in host breadth (Janz *et al.* 2001, Janz & Nylin 2008) - gains of new host lineages are preceded by host breadth expansion (Janz *et al.* 2001) - positive correlation between diet breadth and geographic range (Slove & Janz 2011) - speciation events associated with shifts from generalist to specialist (Hardy, Otto 2014)	- clades including generalist species are more speciose than clades with only specialists (Weingartner, Wahlberg, Nylin 2006) - speciation rates higher in lineages with labile host breadth (Hardy, Otto 2014)
Musical chairs	No prediction	Less conservatism of host plant in more speciose lineages (Hardy, Otto 2014)	Host breadth is not labile (Hardy & Otto 2014; Hardy 2017)	- negative association between speciation rates and host breadth (Hardy, Otto 2014) - speciation rates positively correlate with host switching rates (Hardy, Otto 2014)

264 The comparison of the **diversification dynamics**[*] of both herbivorous insects and their host-
265 plants provide a more direct test of host-driven speciation hypotheses: under host-driven
266 adaptive radiation insect diversification dynamic is expected to roughly follow the
267 diversification of its host-plant lineage. These can be investigated through diversification
268 analyses (see II. 3.2) but can also include the comparison of the fossil records of both insects
269 and plants (Labandeira et al. 1994; Labandeira, Currano 2013). In general, the studies of fossil
270 assemblage are decoupled from phylogenetic studies of plant/insect associations. We advocate
271 for combining fossil and phylogenetic evidence whenever possible.

272

273 **II. 2) Reconstructing the evolutionary trajectory of traits involved in host-plant use**

274   II.2.1) Evolution of host associations

275   Phylogenetic inferences are widely used to reconstruct the evolutionary trajectories of
276 phenotypic traits throughout the diversification of a lineage. Most phylogenetic studies of
277 phytophagous insects map the history of host association onto the resulting trees –at different
278 taxonomic levels (host plant order, family, genus depending on the level of host specialization
279 of the insect clade studied, multistate characters are used when species are polyphagous).
280 These reconstructions generally suggest a pattern of **host conservatism***. This assertion stems
281 from mere observations of the reconstructions but numerous studies conduct statistical tests.
282 These include the permutation tail probability test (PTP; (Faith, Cranston 1991) or some index
283 of phylogenetic signal such as (Pagel 1999) lambda (Λ) (see Winkler, Mitter 2008 for a review
284 and Leppanen et al. 2012; Wilson et al. 2012 for more recent studies).

285   Host conservatism is often interpreted as following the predictions of "*Escape and*
286 *Radiate"* and therefore evidence that speciation was promoted by host-plant specialization.
287 However, showing that related insects feed on related plants does not say much about the
288 process that has generated this pattern nor connects mechanistically host-plant use evolution
289 to speciation. The use of vague wording such as host-associations *favour* or *constrain*
290 speciation is commonly found when discussing host-conservatism in the literature and it is
291 difficult to conclude from these studies that specialization towards one or a few plants species
292 is the main pathway towards the formation of new phytophagous insect species. The pattern
293 of "*host conservatism*" is in agreement with a scenario in which insects have radiated onto a
294 plant lineage but it could also suggest that host-plant shifts are not important promoters of
295 speciation events.

296   A more direct estimation of the contribution of host-plant adaptation in the speciation
297 process consists in inferring the frequency of host-plant shifts in relation to speciation events.

If adaptation to different ranges of host-plants drives reproductive isolation and speciation, it follows that insect **sister species*** should partition host-plant resources: i.e. they should show no or little overlap in the plant species they use.

To test this hypothesis, early studies have conducted sister species comparisons of host ranges and more recent studies have reconstructed the evolution of insect ecological niches (defined as the combination of feeding habits and host-plant species or families) and estimated the number of niche shifts associated with speciation events (Table 2.1). Comparisons of alternative models of evolution of host-use have also been conducted All studies but one showed that the numbers of niche shifts observed generally represented less than 50% of the speciation events and were generally lower than expected if the niches were randomized onto the phylogeny. This suggests that ecological speciation is not the main process behind the diversification of these lineages. Authors have also observed that host-use differentiation occurred at the root of the trees and therefore concluded that it played a minor role in recent speciation events (Table 2.1).

We must keep in mind that these methods probably overlook many host shifts (shifts that resulted in population extinction). Therefore, the studies that estimated niche differentiation at speciation events probably represent an overestimation of the impact of host shifts in speciation. It is nevertheless surprising that such studies have not been conducted on more study systems. This is likely due to the fact that they require a precise knowledge of the range of host-plants used by each insect species.

II.2.2) Host-breadth evolution

In order to test the predictions of macroevolutionary scenarios and investigate the role of specialization in insect species formation, phylogenetic studies have also investigated the distribution of host breadth throughout the evolutionary history of insects groups.

According to the "*Oscillation hypothesis*", host breadth should vary along the phylogeny of insects and the character state "generalist" should be transient; i.e. the phylogenetic reconstruction should show many transitions between specialists and generalists. Many studies have indeed demonstrated the lability of host breadths (Table 2.2), which is compatible with the *Oscillation* hypothesis but does not necessarily prove it.

In order to test whether these changes are linked with speciation, some studies have investigated how gains and losses of new hosts along the phylogenetic history of lineage relate to the number of speciation events -obtaining alternative results on Nymphalidae (Table 2.3).

Table 2: Summary of studies testing the predictions of host-driven speciation scenarios using phylogenetic methods. Cospeciation studies and studies on host-plant conservatism are not included as they are already synthetized in respectively: de Vienne *et al.* 2013 and Winkler & Mitter 2008.

Predictions tested	Taxa	Reference	Approaches	Results	Conclusions
1) Partitioning of host plants at speciation events	*Aphanartum* (25 spp.)	Jordal & M Hewitt 2004	Sister species comparison of host ranges	Ø	Geographic isolation is more important than host switching in speciation events
	Nematinae (125 spp.)	Nyman *et al.* 2010	Investigate niche shifts at speciation nodes through ancestral state reconstruction	Ø	
	Blepharoneura (49 spp.)	Winkler *et al.* 2018		Ø	
	Cinara (76 spp.)	Jousselin *et al.* 2013		Ø	
	Caloptilia (13 spp.)	Nakadai & Kawakita 2016	Niche dissimilarity index through time	Ø	
	Neodiprion (19 spp.)	Linnen & Farrell 2010	Comparisons of models of evolution of host use (speciational model vs gradual)	√	Host shift (in allopatry) induces speciation
2) Host breadth is labile	*Dendroctonus* (19 spp.)	Kelley & Farrell 1998	Phylogenetic signal of host breadth	√	Repeated broadening of host breadth
	Lymantrinae (55 genera)	Wang *et al.* 2017	Phylogenetic signal of host breadth and transition rates estimation	√	Oscillation
	Nymphalidae (551 spp.)	Nylin *et al.* 2014	Phylogenetic signal of host breadth and transition rates estimation	√	
	Nymphalini (31 spp.)	Janz *et al.* 2001	Visualization of reconstruction of host breadth	√	
	15 insect groups	Nosil & Mooers 2005	Estimation of transition rates in host breadth	√	Specialization is not a dead-end
	Boloria (37 spp.)	Simonsen *et al.* 2010	Visualization of reconstruction of host breadth	√	Repeated broadening of host breadth, **oscillation**
	Nymphalidae	Hamm & Fordyce 2016	Phylogenetic signal of host breadth	Ø	**No oscillation**
3) Changes in host breadth spur diversification or speciation events are associated with shift away from polyphagy	Papilionoidea (2573 spp.)	Hardy & Otto 2014	Use of BiSSEness to test whether change in host breadth is associated with speciation events (speciational model vs gradual model of evolution for host breadth)	Ø	**Musical chairs**
	Nymphalini (31 spp.)	Janz *et al.* 2001	Estimate whether polyphagy is ancestral, estimate gains of new host vs losses of hosts (if polyphagy drives speciation; gains should exceed losses)	√	**Oscillation**
	Nymphalini (172 spp)	Hardy 2017	Use of DEC model to reconstruct the history of host use and estimate gains and losses of plants	Ø	Speciation not associated with shift away from polyphagy, **no oscillation**
4) Detoxification mechanisms in insects evolve in response to plant association	Blepharidae (23 spp.)	Becerra 1997	Comparison of insect phylogeny with chemical defense similarity dendrogram of host plants	√	Coevolutionary arm race
	Blepharidae (37 spp.)	Becerra 2003	Comparison of timing of acquisition of plant defenses and insect counter-defenses	√	Coevolutionary arm race

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	Blepharidae (37 spp.)	Becerra 2003	Comparison of timing of acquisition of plant defenses and insect counter-defenses	√	Coevolutionary arm race
	Lygaeinae (20 spp.)	Bramer *et al.* 2015	Reconstruction of the ability to resist or sequester cardenolides	√	Adaptation of insects to host plant defenses
	Heliconius	de Castro *et al.* 2018	Review on correlated evolution of anti-herbivory adaptations in plants and counter-adaptations in *Heliconius*	√	Adaptation of insects to host plant defenses
	Melitaeini (77 spp.)	Wahlberg 2001	Reconstruction of host association on insects and the presence of glycosides in associated plants	√	Insects switch to chemically similar plants
	174 taxa	Endara *et al.* 2017	Comparison of Lepidoptera assemblages associated with different plant species	√	Similarity of assemblages on chemically similar plants; host association driven by similarity of plants
	Apocynaceae	Livshultz *et al.* 2018	Evolution of cardenolide production in plants	√	Plant defenses evolve in response to herbivorous insect predation
5) Diversification rates in phytophagous insect clades > non phytophagous insects	13 families in various orders	Mitter *et al.* 1988	Sister groups comparison of phytophagous vs non phytophagous clades	√	Phytophagy promotes diversification
	1900 spp	Hunt *et al.* 2007	Sister groups comparison of phytophagous vs non phytophagousclades and estimation of diversification rates	Ø	Various types of niche shifts explain beetles diversification
	Erolytidae, 53 taxa	Leschen & Buckley 2007	Correlated evolution between species richness and phytophagy	Ø	
	367 spp, 172 families	McKenna *et al.* 2015	Infer shifts in diversification rates (MEDUSA)	√ & Ø	Some shifts associated with phytophagy others not
	31 insect orders	Wiens *et al.* 2015	Phylogenetic regression	√ & Ø	Different ecological factors prevail at different scales: phytophagy promotes diversification overall but not in all orders
6) Major host shifts spur diversification	Nymphalidae (54 genera)	Nylin & Wahlberg 2008	Estimation of diversification rates after two major host shifts	√	**Escape and radiate and/or Oscillation**
	Butterflies (15 groups)	Fordyce 2010	Test for shifts in diversification rates across the phylogeny (LASER)	√	Burst of diversification concomitant to some host shift, **Escape and radiate**
	Adelpha (38 spp.)	Mullen *et al.* 2011	Test for shifts in diversification rates across the phylogeny (SymmeTREE)	√	One rate shift attributed to hots shift but also other ecological factors
	Adelpha & *Limenitis* (200 spp.)	Ebel *et al.* 2015	Test for shifts in diversification rates across the phylogeny (BAMM)		Shift to Rubiacea played a role in insect diversification
	Nymphalidae (398 genera)	Pena & Espeland 2015	Detection of shifts in diversification rates (MEDUSA & BiSSE with trait= feeding on a particular plant family)	√ & Ø	Shift to Solanacea spurred diversification
	Hesperidae (290 genera)	Sahoo *et al.* 2017	Detection of shifts in diversification rates (LASER), BAMM, BiSSE (trait= feeding on monocotyledonss vs dicotyledons) & HiSSE	√	Several diversification shifts however could be associated with grassland expansion and geographic factors

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7) Acquisition of detoxification mechanisms spur diversification in insects, new defenses spur diversification in plant	Pierinae (60 spp.)	Wheat *et al.* 2007	Distribution of a defense mechanism in insect and assessment of the glucosinolate in their host / comparison of rates of diversification in two sister clades with and without this defense	√	**Escape and Radiate**
	Pieridae (96 spp.)/Brassicales	Edger *et al.* 2015	Distribution of of a defense mechanism and estimation of shifts in diversification rates in plants and insects (MEDUSA)	√	Complex coevolutionary arm race involving gene duplication in plants and associated with shift in diversification
	Asclepias	Agrawal *et al.* 2009	Comparison of various model of evolution for plant defences including a speciational model	√	Plant defenses are costly and can hinder diversification
8) Adaptive radiation pattern	*Erebia* (74 spp.)	Pena *et al.* 2015	Test for shifts in diversification rates (BAMM & DDD)	√ & Ø	Density dependant model fits the data, diversification shifts not always significant depending on methods : acceleration of diversification associated to colonization of new area
	Cinara (92 spp.)	Meseguer *et al.* 2015	Test for shifts in diversification rates (TreePar)	Ø	Constant rate of diversification, no adaptive radiation
	Blepharoneura (49 spp.)	Winkler *et al.* 2018	Test for shifts in diversification (LASER, DDD)	Ø	Constant rate of diversification, no adaptive radiation, diversification patterns mostly explained by geographical factors
	Sesamiina (241 spp.)	Kergoat *et al.* 2018	Test for shifts in diversification through different methods (BAMM, *SSE)	Ø	Inverse patterns of diversification in insects and associated plants ; host plant diversity alone does not explain insect diversification
9) Positive relationship between insect lineage diversity and their host plant diversity	115 spp.	Farrell & Mitter 1998	Sister-clade comparison of angiosperm vs non angiosperm feeding groups	√	Host- driven speciation
	Nymphalidae (309 genera)	Janz *et al.* 2006	Sister clade comparisons	√	**Oscillation hypothesis**
	Lymantrinae (55 genera)	Wang *et al.* 2017	Phylogenetic regression	√	
	Coccidae (158 genera)	Lin *et al.* 2015	Regression without phylogenetic correction	√	Host- driven speciation
	Cecidomyiid (352 genera)	Joy & Crespi 2012		√	

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10) Clades including generalists speciate faster than clades with only specialists; diversification rates are positively correlated with host breadth	*Nymphalis* & *Polygonia* (20 spp.)	Weingartner *et al.* 2006	Sister clade comparison of species richness between clades with only specialist and clades that encompass species with large host breadth	√	Broadening of host range: plasticity facilitates the capture of new hosts and subsequent ecological speciation
	Nymphalidae (54 genera)	Nylin & Wahlberg 2008	Estimation of diversification rates	√	Clades experiencing higher rates of diversification experience a polyphagous state: **oscillation**
	Papilionoidea (2573 spp.)	Hardy & Otto 2014	⎫	Ø	**No oscillation, Musical chairs**
	Nymphalidae	Hamm & Fordyce 2015 Hamm & Fordyce 2016	⎬ Test for shifts in diversification through different *SSE methods (trait= host breadth)	Ø	Host breadth dynamics does not drive diversification
	Coccidea	Hardy *et al.* 2016	⎭	√	Specialization by drift
	Nymphalini (172 spp.)	Hardy 2017	Phylogenetic independant contrasts (proportion of generalists vs number of species in a genus)	Ø	**No oscillation**
	Sesamiina (241 spp.)	Kergoat *et al.* 2018	Evaluate whether shifts in diversification rates follow shifts in host breadth (BAMM and others)	Ø	**Oscillation** might not be detectable at this scale
11) Diversification rates of clades with labile host association> diversification rates of clades with conservatism in host association	Papilionoidea (2573 spp.)	Hardy & Otto 2014	Use of *SSE to test whether speciation rates vary between lineages, correlation between host switching rates and diversification rates	√	**Musical chairs**

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Coleoptera ,   Lepidoptera,   Hemiptera,   Diptera,   Hymenoptera
√ Prediction verified, Ø prediction not verified

DEC (Ree, Smith 2008); Laser (Rabosky 2006); Medusa (Alfaro et al. 2009) , BiSSE (Maddison, Midford, Otto 2007), BAMM (Rabosky et al. 2014), DDD (Etienne et al. 2012), HiSSE (Beaulieu, O'Meara 2016), SymmeTREE (Chan, Moore 2002)

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337   Maybe another way to investigate the link between 'host-breadth changes' and 'gains
338 of new hosts' without using diversification analyses would be to test for the correlated
339 evolution of these two characters; i.e. explore how often the gain of new hosts is associated
340 with a transition from specialist to generalist.

341   II.2.3) Evolution of traits involved in host-plant choice

342   Several studies that aimed at finding support for the coevolutionary arm race
343 hypothesized by the *Escape and Radiate* scenario have investigated the evolution of
344 detoxification mechanisms in insect lineages and showed that they correlate with changes in
345 their host plant defences (Table 2.4). Studies that show that host switches occurred between
346 plants with similar defenses, rather than closely related plants generally conclude to
347 coevolutionary arm race between insects and associated plants. However some of these
348 studies argue in favour of a scenario in which herbivores simply "choose" host-plants based
349 on their own defensive traits. Host switches underlie insect species differentiation but this
350 differentiation is not the product of coevolutionary interactions.

351   Reconstruction of the history of plant defences was also conducted on plant
352 phylogenies. However direct tests of acceleration of diversification upon the acquisition of
353 new defence mechanisms sometimes contradict the '*Escape and Radiate*" prediction and
354 suggests that investment in costly defences can impede plant diversification (Table 2.4).

355   In contrast to detoxification mechanisms, traits involved in host recognition and host-
356 plant choice (chemosensory traits) have been less studied in a phylogenetic context. However
357 their evolution probably plays as important a role as adaptations to plant defences in
358 phytophagous insect speciation (Smadja, Butlin 2009). Matsuo (2008) showed that an odour
359 binding protein can evolve relatively fast in closely related *Drosophila* species through gene
360 duplications and losses, and proposed that this dynamic could explain the evolution of host
361 preferences in this species complex. Comparative genomic analyses of odour binding protein
362 and chemosensory proteins from the genomes of several Arthropoda species (Sánchez-Gracia,
363 Vieira, Rozas 2009; Vieira, Rozas 2011) revealed a high number of gains and losses of genes,
364 pseudogenes, and independent origins of gene subfamilies. This dynamic, if analysed in
365 relation to host choices and host breadth in a phylogenetic context, could explain some host
366 shifts and subsequent speciation events. Finally, deciphering the evolutionary dynamics of
367 genes involved in mate recognition and their link with host association could also inform us
368 on the role of host-plants in the speciation of insects that feed (and often mate) on them. For
369 instance, Schwander et al. (2013) showed that cuticular hydrocarbon profiles involved in mate

370   choices vary among *Timema* species (Phasmatodea), and that most evolutionary changes in
371   hydrocarbon profiles occur in association with host-plant shifts and speciation events in this
372   genus of phytophagous insects.

373   In summary, many studies have investigated the evolution of detoxification
374   mechanisms in insects framing their hypotheses within the ***Escape and Radiate*** theory.
375   Studies investigating changes in traits involved in host recognition throughout the
376   diversification of insects are rare and they are generally not framed within host-plant driven
377   scenarios. Such studies are needed in order to determine which traits underlie host-plant
378   associations and whether their evolution drives speciation events (see Perspectives).

II. 3) Studying how host-plants use impact the diversification dynamics of herbivorous insects

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381   Methods for testing diversification dynamics have expanded over the last decade.
382   Testing for the existence of temporal bursts of diversification was once restricted to analyses
383   of groups with comprehensive fossil records. Diversification dynamics can now be studied
384   through comprehensive phylogenies (Goswami, Mannion, Benton 2016). Given the breadth of
385   available methods, theoretically, nearly all hypotheses can be put to test.

386   II.3.1) Are phytophagous insects more diversified than their related counterparts?
387   Studies that posit that host-plant adaptation favours phytophagous insect diversification
388   predict that those are much more diversified than non-phytophagous insects. However this
389   assertion deserves to be statistically tested. The first study addressing this question suggested
390   that herbivorous clades contain more species than their non-phytophagous sister clades (Table
391   2.5). However, comprehensive phylogenies associated with recent comparative methods
392   suggest that several types of niche shifts beside phytophagy can explain insect species
393   richness. Studies within insect orders have mostly been addresses within Coleoptera –as they
394   are by far the most diverse order, but more in-depth analyses of other orders would also be
395   insightful.

396   To our knowledge likelihood-based character state dependent diversification models (known
397   as, the *SSE models, such as BiSSE, ClaSSE and QuaSSE for binary, multistate and
398   quantitative traits, respectively Maddison, Midford, Otto 2007; FitzJohn 2010; Maddison,
399   FitzJohn 2015), which test whether transition in character states are associated with variations
400   in speciation and extinction rates, have not been yet used to test the role of phytophagy in
401   insect diversification. A robust estimate of diversification parameters by these methods
402   requires dense and random species sampling throughout the phylogeny (more than 15% of the

403   species must be included in the phylogeny in order to conduct unbiased tests; FitzJohn 2010).

404   Therefore *SSE tests await the availability of comprehensive phylogenies in more insect

405   orders. Nevertheless, a caveat of these diversification models is that they may overlook more

406   complex models involving many unmeasured and co-distributed traits. In particular, for

407   phytophagy, other traits that may drive shifts in diversification may cause a spurious detection

408   of increased diversification rates in phytophagous insects if this trait's states partly correlate

409   with phytophagy, or may instead erase any signal due to phytophagy. The HiSSE model

410   (Beaulieu, O'Meara 2016) which models hidden characters that influence diversification

411   might help untangling these confounding factors.

412   <u>II.3.2) Testing for adaptive radiation</u>

413   Diversification analyses can also be applied to test whether the capture of new host-

414   plants has favoured adaptive radiations. Under such a scenario, the diversification curve of

415   phytophagous insect clades should exhibit early bursts of speciation upon the capture of new

416   groups of host-plants. Insect lineages are eventually expected to fill the newly found niche

417   space and the diversification curves should reach a plateau.

418   Several studies have investigated acceleration in speciation rates in insect lineages

419   upon the capture of new host-plant lineages (Table 2.6) or detoxification mechanism (Table

420   2.7). These studies have mainly been conducted in butterflies. Studies have rarely included

421   trait-dependent diversification models and when they did, they often relied few colonization

422   events that were also correlated with important geographical changes, making it difficult to

423   conclude on the causal effect of the sole host shifts (Table 2.6).

424   Among studies testing adaptive radiation patterns (Table 2.8), few have investigated

425   whether the number of species reached a plateau after an initial burst of speciation. This is

426   better addressed using density-dependent models of diversification (DDD) that test whether

427   rates of speciation decrease once the number of species supposedly reach the carrying

428   capacity of the novel ecological niche (Rabosky, Lovette 2008; Etienne et al. 2012). But these

429   tests can only be applied to lineages in which the number of species in each subclade is well

430   known. In any case, studies generally provided weak support for adaptive radiation scenarios

431   (Table 2.8). They all suggested that diversification patterns cannot solely be explained by the

432   availability of suitable host-plants and suggested that abiotic factors such as geography and

433   temperature should be taken into account in diversification scenarios.

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436   <u>II.3.3) Correlating host breadth with diversification dynamic</u>

437   Advocates of the *Oscillation hypothesis* suggest that clades showing a higher diversity

438 of host-use (using more host-plant species altogether) should be more diverse than their sister

439 clade (Janz, Nylin, Wahlberg 2006), and this prediction has been verified in butterflies,

440 Coccidae and some gall inducing flies (Table 2.9).

441   Although these results support a model where the diversity of phytophagous insects is

442 sustained by the diversity of the hosts they use, they could fit both a model of *Oscillation* and

443 the *Musical chairs* hypothesis. The latter indeed predicts that lineages including specialist

444 species that often switch between hosts use a large number of host-plants. To tell apart the

445 *Oscillation* from the *Musical chairs*, it is actually more informative to test how host breadth

446 variations affect insect diversification dynamics. According to the *Oscillation* hypothesis,

447 clades including generalist species should be more speciose than clades including only

448 specialists. Early papers have used fairly basic methods such as 'sister clade analyses" to test

449 this prediction while more recent investigations have adopted trait-dependant diversification

450 models (Table 2.10). These studies provided mixed support for *Oscillation* scenarios. But

451 even when a specific prediction was met, the authors could not always reach a firm

452 conclusion. As previously mentioned, the transient nature of the generalist feeding diet under

453 host-driven speciation scenarios makes it difficult to derive clear predictions. The negative or

454 positive relationship observed between host breadth dynamics and diversification can always

455 be obscured by the rapid evolution of host breadth (Nylin, Janz 2009; Janz et al. 2016). The

456 way host breadth is measured (binary vs continuous) is also known to affect the results

457 (Hamm, Fordyce 2015) and it has been long recognized that categorizing species into either

458 specialist or generalist can be somewhat subjective (Janz, Nyblom, Nylin 2001). Furthermore,

459 although the musical chair yields specific predictions, those are mainly rebuttals of the

460 "*Oscillation*" predictions (but see Table 2.11). But rejecting an "*Oscillation*" scenario does

461 not instantly mean that a "*Musical chairs*" scenario is at play. Hence despite the existence of

462 sophisticated methods and comprehensive phylogenies, the prevalence of these scenarios in

463 insects is still debated.

464   Another caveat of these studies lies in the distinction between speciation and

465 extinction rates. Indeed, if specialization towards host plants can accelerate speciation it can

466 also increase extinction risks when plants are not highly abundant. Consequently, predicting

467 exactly how changes in host-plant breadth affect diversification rates is difficult. Finally

468 diversification methods such as *SSE models are known to generate false positives

469 (Bouchenak-Khelladi et al. 2015; Rabosky, Goldberg 2015; Davis, Midford, Maddison 2013),

470 and can thus inflate the role of host plants and host breadth in diversification when those are
471 investigated. It is therefore, highly recommended to conduct the analyses on a set of
472 randomized trees in order to test whether the constrained diversification model (i.e. the model
473 in which evolutionary transitions in character states are associated with shifts in extinction
474 and/or speciation rates) is also chosen in these analyses (as in Hardy, Peterson, Normark
475 2016).

476   Moving away from methodological issues, our survey also underlines that many
477 studies that explore macroevolutionary scenarios have been conducted on Lepidoptera. In
478 order to have a better understanding the role of host-plant shifts in insects diversification, it
479 seems necessary to test the predictions of macroevolutionary scenarios on more insect groups.
480 Aphids (Hemiptera) could be good candidates for such investigation. Their range of host-
481 plants is vwell documented (Blackman, Eastop 2006; Holman 2009). Although most aphids
482 are host-specific, there are some polyphagous species. Some species are even only
483 polyphagous during part of their life-cycle. This temporary broadening of diet has already
484 been suggested to facilitate the capture of new host-plants (Moran 1992) and subsequent
485 speciation onto these new lineages. These life-cycle transitions could have thus favoured the
486 diversification of this clade (Moran 1992; Jousselin, Genson, Coeur d'Acier 2010). This
487 scenario actually fits the *Oscillation hypothesis*. Other Hemiptera which host plant repertoire
488 is well known such as Coccidae (Garcia-Morales *et al.* 2016) and psyllids (Ouvrard *et al.*
489 2015) could also be used to test the predictions on the role of host breadth evolution in
490 macroevolutionary scenarios. The limitation for these groups for now lies in the availability of
491 robust and comprehensive phylogenies.

III Perspectives

493   As seen throughout this review, phylogenetic comparative methods provide the
494 template to test hypotheses on the role of host plant association in the speciation of
495 phytophagous insects. While those methods have undoubtedly advanced the field significantly
496 since "the *Escape and Radiate*" paper, readers must keep in mind that phylogenetic
497 comparative methods often rely on mere correlations. Significant associations between
498 character changes and the cladogenetic events might arise as a consequence of speciation
499 itself when post-speciational character changes occur. Furthermore, comparisons of models of
500 evolution such as those used in trait-dependent diversification analyses often rely on trees that
501 encompass few transitions in character states and are therefore not always robust (see
502 Beaulieu, Donoghue 2013). In such analyses, the "best model" is not necessarily the true

model and significant *P* values should not be interpreted as strong evidence for an evolutionary scenario. Finally, all these methods are sensitive to sampling biases and those need to be carefully taken into consideration.

In addition to using the approaches focused on host plant associations and diet breadth cited throughout this review, one way to further investigate hypotheses of speciation driven by associations with host-plants would be to integrate a variety of data in a phylogenetic context. Below we outline three potential directions for future research: 1) disentangling the role of plant-insect interaction from that of co-variates, such as geography and climate; and 2) studying traits and genes underlying the association; 3) combining phylogenetic analyses with interaction network approaches including other partners, at various ecological scales (from community-scale to global scale).

III.1) Investigating the role of abiotic factors: geography and climate

Geography and ecology are always closely intertwined in speciation scenarios. There have been several studies that have investigated geographic range expansion in herbivorous insects (Becerra, Venable 1999; Slove, Janz 2011); climate induced host shifts (see Winkler, Mitter, Scheffer 2009; Nyman et al. 2012 for a review and recent studies since then Sanchez-Guillen et al. 2016; Lisa De-Silva et al. 2017; Owen et al. 2017; Pitteloud et al. 2017; Sahoo et al. 2017) and climate driven diversification dynamics (Kergoat et al. 2018). All these studies suggest that abiotic factors are entangled with host-plants changes in species diversification scenarios. However there are few studies that explicitly test the predictions of speciation through geographic isolation (Barraclough, Vogler 2000) and whether these events systematically accompany host shifts or sustain most speciation events (but see Jordal & Hewitt 2004; Jousselin et al. 2013; Doorenweerd, van Nieukerken, Menken 2015; Hardy, Peterson, Normark 2016). Such analyses are important if we want to tell whether adaptations to new host plants represent post-speciational changes following geographic isolation rather than the main driver of speciation events. Cospeciation methods that take into account the biogeographic history of interacting lineages (Berry *et al.* 2018) could be also used to investigate whether host shifts are associated with dispersal events in systems where hosts plants and insects phylogenies show some congruent patterns.

III.2) Unravelling traits involved in the interaction and their underlying genes, and integrating this information in phylogenetic studies

Interactions between insects and their host-plant are ultimately mediated by traits, such as host-plant defences and the capacity of circumventing plants defences, but also host-plant cues and the capacity for herbivores to detect those cues. Characterizing such traits, their genetic determinism and looking at their evolutionary trajectory would greatly advance our understanding of the diversification of insects (*e. g*., de Castro et al. 2018). Testing whether different trait states are associated with different speciation rates can be performed using *SSE methods (*e. g.*, as in Onstein et al. 2017 for a trait related to frugivory in palm trees). In addition, methods that test whether patterns of trait evolution conform to a model accounting for interactions mediated by those traits are currently being developed (Manceau, Lambert, Morlon 2016; Drury et al. 2017), and they could inform on the processes underlying herbivore diversification. However, targeting traits involved in plant-insect interactions may be challenging. Pivotal traits are difficult to identify, they include chemical, behavioral and metabolic traits and when they are properly characterized they are often multigenic.

Perhaps a promising direction for future research is the implementation of a hybrid genomic approach that combines transcriptomics, phylogenomics, comparative analyses and population genomics (see Nevado et al. 2016). In such approaches, full transcriptomes of species from a target clade (for instance, a clade of phytophagous insect) are generated. These transcriptomes are used to generate a phylogeny, where classical diversification and character evolution tests can be performed (evolution of characters, test for diversity-dependent diversification, shifts in diversification following host-plant shift). Then, genes under selection can be detected from transcriptomic data using population genetics statistics, and can be matched to existing databases (e. g., Lepbase for Lepidoptera, Challis *et al.* 2016) for identification purpose. Additionally, genes that are down or upregulated can also been detected by classical tests of differential expression and identified, and the association of genes under selection, either via different sequence or expression pattern, with species diversity can be tested. One of the limits of this approach lies in the availability of specimens (transcriptomic data need to be obtained from fresh or suitably preserved tissues; biological replicates are needed), those might be difficult to obtain throughout an entire phylogenetic tree. The availability and quality of the reference gene database to match genes with putative functions might also limit the applications of this approach.

III.3) Combining phylogenetic with interaction network approaches, at various ecological scales

567   Herbivores and the plants they feed on form interaction networks, and as such the
568   structures of the networks can be characterized by several parameters, such as modularity (the
569   propensity of a group of species to interact with a similar set of partners) and nestedness (the
570   propensity of specialist species to interact with generalist species and vice-versa).
571   Antagonistic interaction networks, such as plant-herbivore networks, tend to be highly
572   modular (Thébault, Fontaine 2010). A recent study combining interaction network with
573   phylogenetic approaches on simulated and real datasets predicted that the *Escape and radiate*
574   scenario should produce a modular network structure, whereas the *Oscillations* scenario
575   should produce a more nested structure (Braga et al. 2018). When applied to real data (two
576   butterfly families, Nymphalidae and Pieridae), this approach revealed that host-plant butterfly
577   networks tend to be both modular and nested, which the authors interpret as being the result of
578   a complex pattern of diversification, involving both episodes of radiation on new hosts
579   (producing modules containing closely related species) and occasional shifts to other host
580   lineages, producing both nestedness within modules and connections between modules.
581   Additionally, phylogenetic and network approaches could be expanded to encompass other
582   interacting partners (*e. g.* Elias, Fontaine, van Veen 2013; Ives, Godfray 2006) . Indeed,
583   insect-host-plant communities can be seen as ecosystems where biotic interactions, such as
584   parasitism and mutualism also take place (Forister et al. 2012). These other partners can
585   indirectly influence the interaction between plants and their herbivores: *e.g.*, direct
586   competition (Jermy 1988) apparent competition between herbivores, stemming from shared
587   natural enemies, (Holt 1977), and vice-versa (*e. g.,* when herbivory elicits anti-herbivore
588   defences mediated by herbivore enemies, Fatouros et al. 2008). Multitrophic interactions
589   probably explain many diversification patterns in herbivorous insects (Singer, Stireman 2005)
590   Finally, such approaches could be applied both at a large scale (*e. g.*, Braga et al. 2018), to
591   embrace global patterns of diversification and interaction, or at the community level (Elias,
592   Fontaine, van Veen 2013; Ives, Godfray 2006), where interactions actually occur, and where
593   fine-scale processes (*e. g.*, host-plant shift at the species or the population level) can be
594   unveiled.

Conclusions

596   Many phylogenetic studies of plant-insect associations now include formal tests of
597   macroevolutionary scenarios involving host-driven speciation. In an attempt to summarize the
598   literature on this topic, we show that the predictions of host-plant driven speciation are not
599   straightforward and can vary depending on studies. We advocate a standardization of these

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600   predictions to facilitate cross study analyses. Furthermore, it is also recognized that different
601   scenarios can leave the same phylogenetic signature (Janz *et al.* 2016) and that depending on
602   the analytical approaches undertaken to test the predictions laid out in Table 1, conclusions
603   can vary (Table 2). Unfortunately this means that the interpretations of phylogenetic
604   inferences can remain somewhat subjective. But these shortcomings should not obscure the
605   progresses that have been made in the field. Phylogenetic comparative analyses help framing
606   hypotheses and clarify some of the narratives used to explain the diversification of
607   phytophagous insects. In order to move towards a standardization of phylogenetic approaches,
608   we propose here a (non-exhaustive) list of relatively simple tests that could be applied to an
609   insect phylogeny that includes robust data on host plant association to test some of the non-
610   controversial predictions of host-driven speciation scenarios (Fig. 2). The limitations of these
611   approaches (sampling biases, false positives) have been described throughout this review.
612   Finally, this survey of the literature shows that: 1) the simple assumption that phytophagy has
613   accelerated insect diversification is not always sustained by meta-analyses; 2) the expectation
614   that sister lineage will use different ranges of host plants is not often tested, and, when it is,
615   the predictions of a host-driven speciation scenarios are not always met. We then underline
616   that the results of phylogenetic comparative methods cannot be interpreted as hard evidence
617   as they remain mere correlations. In the end, a full understanding of the processes explaining
618   the diversification of phytophagous insects will require the integration of phylogenies with
619   other data sources and analytical methods.

620   To conclude, if the last two decades have seen the rise of molecular phylogenies and
621   the development of analytical methods that include ecological data, this should not obscure
622   the fact that these data need to be thoroughly curated before any phylogenetic comparative
623   analyses. Qualifying host associations of insect species necessitates field work and advanced
624   taxonomy, as mistakes can seriously impact the results of macroevolutionary studies.
625   Functional studies aimed at deciphering host-plant adapted traits in insects (and in particular
626   traits implied in host choice) and characterizing genes that underlie these traits are also
627   needed to integrate this data in a phylogenetic context and link microevolutionary processes
628   with macroevolutionary scenarios.

a) Host-plant range history
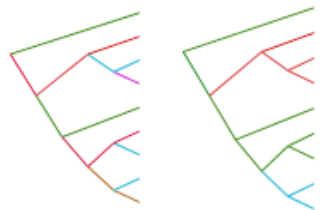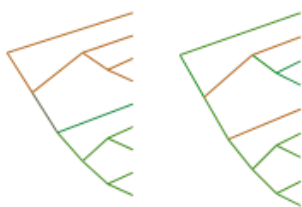

Range 1
Range 2
Range 3
Range 4

H1: Speciation driven by host shifts

H2: Speciation not driven by host shift

Tests: evaluate the number of host shifts and compare models of evolution for host range .

Under H1: number of host shifts ≈ number of speciation events > number expected when host use is randomized; host-plant range follows a speciational model of evolution.

Under H2: host plant range exhibits a phylogenetic signal.
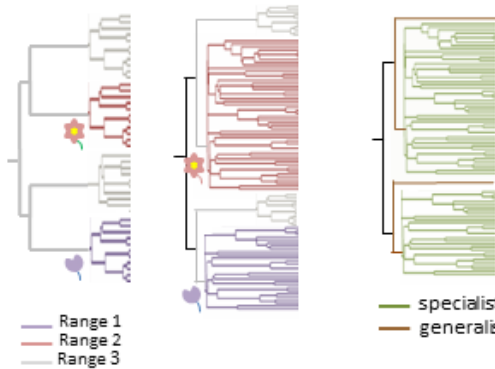
b) Host breadth history

specialist
generalist

H1: No oscillation   H2: Oscillation

Tests: evaluate phylogenetic signal and compare models of evolution for host breath .

Under H1 : host breadth exhibits a phylogenetic signal.

Under H2 : host breadth follows a speciational model of evolution.

c) Diversification analyses

Range 1
Range 2
Range 3

specialist
generalist

H1: No adaptive radiation

H2: Adaptive radiation driven by host shifts (Escape and radiate)

H3: Adaptive radiation driven by shifts in diet breath (Oscillation)

Tests: use sister-clade analyses, PGLs, diversity dependant models of diversification or *SSE models to test the effect of host shift, host breadth and host plant lineage diversity on speciation.

Under H1: no significant shifts in speciation rates upon host shifts.

Under H2: significant shifts in speciation rates upon host shifts, number of insect species higher in species rich plant clades.

Under H3: speciation rate increase following shift away from polyphagy

629
630 Figure 2: Suggestions of phylogenetic comparative methods that can be deployed to test the prediction of host-driven speciation scenarios. a) Tests that rely on a robust reconstruction of host-plant
631 range evolution. b) Tests that rely a robust reconstruction of host-breadth. c) Diversification analyses that rely on reconstruction of both these characters. H1, H2, H3 are
632 alternative scenarios and are represented by a schematic phylogenetic reconstruction above.

Glossary:

Adaptive radiation: the evolution of ecological and phenotypic diversity within a rapidly multiplying lineage (Schluter 2000). It occurs when natural selection drives divergence of an ancestral species into descendants that exploit different ecological niches.

Coevolution: reciprocal evolutionary changes occurring in two or more species that result from reciprocal selective pressures exerted by the interacting partners.

Coevolutionary diversification: when diversification patterns arise from coevolution.

Cospeciation: simultaneous speciation events in lineages involved in long-term interspecific associations which result in congruent phylogenies and temporal congruence of speciation events.

Diversification dynamic: rates of species formation and extinction through time.

Ecological specialization: when species are limited to a restricted set of resources (diet-habitat-niches), as a result of evolutionary trade-offs.

Evolutionary lability: tendency for a character to change frequently throughout evolution.

Generalists: species that use a wide niche (a wide range of host plants belonging to different lineages in the particular case of phytophagous insects).

Host-plant adaptation: heritable trait that confers a selective advantage on a particular host-plant.

Phylogenetic conservatism: tendency for closely related species to be more similar than expected under Brownian motion evolution.

Phylogenetic tracking: it occurs when a host-dependent species (here a phytophagous insect) diversifies and utilizes niches created by the speciation its hosts (here host-plants), this leads to parallel phylogenetic trees but no temporal congruence of speciation events.

Phytophagous insect: an insect that feeds on any plant organ during whole or part of its life cycle, it excludes pollinators feeding on nectar and pollen but include pollinators that feed on developing seeds (*i.e.* seminiphagous insects).

Specialists: species that use a narrow niche (a restricted set of resources).

660 | **Sister species/ sister clades:** the closest relatives of another given unit (species/ clade) in a
661 | phylogenetic tree.

662

663   **Conflict of interest disclosure**

664   The authors of this preprint declare that they have no financial conflict of interest with

665 the content of this article."

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