

1 **The evo-devo of plant speciation**

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6

7 **Abstract**

8 Speciation research bridges the realms of macro- and microevolution. Evolutionary
9 developmental biology (evo-devo) has classically dealt with macroevolutionary
10 questions through a comparative approach to distantly related organisms, but the field
11 later broadened in focus to address recent speciation and microevolution. Here we
12 review available evidence of the power of evo-devo approaches to understand
13 speciation in plants at multiple scales. At a macroevolutionary scale, evidence is
14 accumulating for evolutionary developmental mechanisms giving rise to key
15 innovations promoting speciation. At the macro-microevolution transition, we review
16 instances of evo-devo change underlying both the origin of reproductive barriers and
17 phenotypic changes distinguishing closely related species. At the microevolutionary
18 scale, the study of developmental variation within species provides insight into the
19 processes that generate the raw material for evolution and speciation. We conclude by
20 advocating a strong interaction between developmental biology and evolutionary
21 biology at multiple scales to gain a deeper understanding of plant speciation.

22

23 **Introduction**

24 Speciation research addresses the evolutionary processes generating the extraordinary
25 diversity of life on Earth as well as the patterns derived from them¹. It bridges the
26 realms of macro- and microevolution, respectively dealing with evolutionary
27 phenomena above and below the species level². This transition is defined by the
28 establishment of reproductive barriers restricting gene flow between populations, the
29 prerequisite for species formation under the biological species concept. Speciation
30 studies have classically focused on genetic and ecological mechanisms, essentially
31 ignoring the role of developmental mechanisms. At the same time, the more recent field
32 of evolutionary developmental biology (evo-devo), which aims to understand the
33 developmental mechanisms of evolutionary change³, has usually dealt with
34 macroevolutionary questions through a comparative approach with distantly related
35 organisms⁴, paying less attention to speciation and microevolutionary processes. A
36 broadening of focus in evo-devo to address recent speciation and microevolution has
37 been advocated⁵⁻⁶. Indeed, new research shows the potential of a multidisciplinary
38 approach including evo-devo to provide deeper insight into speciation.

39 Plants, and particularly the outstandingly diverse angiosperms, provide excellent
40 opportunities for this approach. A number of model systems for the study of speciation
41 have been characterised genetically, developmentally and ecologically. Moreover,
42 several genes involved in developmental processes potentially generating pre- and post-
43 zygotic reproductive isolation have been characterized⁷⁻⁸. Here we review available
44 evidence for the power of evo-devo approaches to understand speciation processes and
45 patterns in plants at multiple scales. Our focus is mainly on evolutionary changes in
46 developmental patterns, or “developmental repatterning”³, underlying reproductive
47 barriers and other phenotypic differences arising during or shortly after speciation.

48 Although it is difficult to quantify the role of developmental changes in reproductive
49 isolation relative to other mechanisms, available data suggest that developmental
50 repatterning is particularly relevant to pre-zygotic barriers, and specifically to those
51 acting at the pre-pollination level. In a review of speciation genes in plants⁸, all
52 examples of speciation genes underlying pre-pollination barriers were involved in
53 developmental processes. These included genes causing temporal isolation and
54 pollinator isolation, which are frequent targets of speciation studies in flowering plants
55 (although it has also been argued that pollinator specialisation can frequently be a
56 consequence, and not a cause, of speciation⁹). Post-zygotic barriers, on the other hand,
57 include mechanisms of hybrid inviability and hybrid sterility⁷⁻⁸ that are not described as
58 developmental repatterning, and therefore are not reviewed here. Pre-zygotic barriers
59 are thought to contribute more than post-zygotic barriers to reproductive isolation in
60 plants⁷, which indicates that developmental repatterning plays a crucial role in plant
61 speciation.

62

63 **Macroevolutionary scale**

64 *Speciation-promoting traits.* Evo-devo research has frequently focused on comparing
65 developmental processes across species separated by large evolutionary distances.
66 These research lines fall within the realm of macroevolution, which is generally
67 regarded as the long-term cumulative result of microevolutionary mechanisms¹⁰.
68 However, some macroevolutionary patterns may not be entirely explained in this way,
69 such as the differential diversification of clades (species selection) and the ways in
70 which such differential diversification is related to morphological variety (disparity)¹¹-
71 ¹². Bursts of speciation (radiation) may be the result of the evolutionary acquisition of
72 “key innovations”. A key innovation can be defined as an evolutionary change in a trait

73 that is causally linked to an increased diversification rate in the resulting clade¹³. This
74 may be the result of exposing the lineage to new areas of phenotypic space and new
75 ecological opportunities^{12,14}. Evo-devo can provide information on the
76 microevolutionary developmental mechanisms by which key innovations first evolved,
77 along with insights into their macroevolutionary effect on speciation patterns.

78 The evolution of many species-rich plant clades was preceded by whole-genome
79 duplication (WGD, or polyploidy) events that allowed the diversification of regulatory
80 genes involved in the development of key innovations¹⁵⁻¹⁶. Frequently, however, there is
81 a lag between innovation and radiation¹⁷. It has been suggested that, after an innovation
82 first appears, strong developmental robustness needs to evolve for natural selection to
83 efficiently explore phenotypic and ecological space, thus leading to both species
84 diversification and an increase in morphological disparity¹². As an outstanding example,
85 the angiosperm flower can be considered a combination of key innovations that fostered
86 diversification through the exploration of a brand-new array of plant-pollinator
87 interactions.¹⁸ Although the developmental origin of the angiosperm flower has not yet
88 been explained, it is known that a WGD event occurred before the diversification of
89 angiosperms, and duplication of homeotic MADS-box genes controlling the identity of
90 floral organs was likely important in the origin of the flower¹⁵⁻¹⁶. A role for
91 developmental robustness in the subsequent radiation is supported by the observation
92 that early-diverging lineages of angiosperms are relatively species-poor and display a
93 low degree of floral developmental robustness, in contrast with the high robustness
94 found in some of the most diverse angiosperms families, such as the Orchidaceae¹².

95 Aside from the actual flower itself, additional floral key innovations independently
96 acquired in multiple families are thought to have consistently enhanced diversification
97 within the angiosperms¹⁴. The developmental mechanisms recruited in these

98 independent origins of key traits may or may not be the same between species at the
99 organ level (e.g. different organ identity, contrasting growth patterns), cell level (e.g.
100 patterns of cell division and cell expansion) or molecular level (e.g. changes in
101 regulatory or coding regions of the same or different genes). Thus, recurrently acquired
102 key innovations constitute phylogenetic replicates that can be used as “metamodels” in
103 which to test the consistency of those developmental innovations promoting speciation
104 across lineages¹⁹. For example, floral zygomorphy (bilateral symmetry) is known to
105 have evolved many times from actinomorphic (radially symmetric) ancestors, and is
106 thought to have promoted speciation by enabling specialized animal pollination²⁰. In
107 eudicots, the evolution of zygomorphy has recurrently involved the recruitment of
108 *CYCLOIDEA*-like genes that are dorsally expressed during flower development²¹. In
109 contrast to zygomorphy, diverse molecular mechanisms might underlie the repeated
110 evolution of nectar spurs, another floral key innovation (Box 1).

111 In addition to key innovations, highly labile traits may be involved in numerous
112 speciation events throughout plant lineages. The best example is probably flower
113 colour, one of the plant traits whose developmental pathways are best understood,
114 particularly those related to anthocyanin pigmentation²². Flower colour is frequently
115 involved in pollinator-driven speciation events in distant angiosperm families²³,
116 providing another useful example of phylogenetic replication at the macroevolutionary
117 scale. In this case, continuing work in multiple models (*Antirrhinum*, *Petunia*,
118 *Aquilegia*, among others; Fig. 1a,b; Box 1) reveals that evolutionary changes tend to
119 occur in “optimally pleiotropic” components of developmental pathways, i.e. those that
120 maximize change in the trait under selection while at the same time minimizing
121 deleterious effects on other traits, such as seed coat development, UV resistance and
122 pathogen defence^{19,24}.

123 *The role of the fossil record.* A source of macroevolutionary information that is
124 frequently overlooked by plant evo-devo is the fossil record. Fossils provide invaluable
125 information about evolutionary patterns that cannot be inferred from extant lineages
126 alone, including calibrations for phylogenetic dating analyses to help estimate the
127 timing of developmental evolution²⁵. A detailed study of the fossil record also allows us
128 to assess historical changes in the disparity (morphological variety) of clades, and their
129 correlation, or lack thereof, with changes in diversity (number of species). Although
130 only preliminary analyses are available for plants²⁶, diversity and disparity seem to be
131 fundamentally decoupled, with maximum disparity frequently being achieved early
132 during diversification of a clade. This is usually followed by an increase in diversity that
133 is not accompanied by further increases in disparity. Proposed explanations for this
134 pattern of diversification through small variations on early evolving themes, also found
135 in animals, include developmental constraints and ecological restrictions. An eco-evo-
136 devo approach integrating the study of fossil and extant lineages may help to distinguish
137 these two non-mutually exclusive hypotheses.

138 Remarkably, fossils can preserve diagnostic features of plant development, and even
139 structural evidence for developmental regulatory mechanisms of extinct plants²⁷⁻²⁹, thus
140 potentially containing information on the origin of evolutionary innovations important
141 for diversification. This is particularly relevant for ancient evolutionary transitions
142 whose signature in extant lineages may be limited. Examples are provided by the
143 evolution of woody growth and tree architecture across plant lineages. Among extant
144 plant lineages, wood is only produced by seed plants. However, the fossil record shows
145 that woody growth has evolved several times in the course of vascular plant
146 diversification, and there is strong developmental evidence that these multiple
147 acquisitions were mediated by a common polar auxin regulatory pathway²⁹⁻³⁰. Evolution

148 of tree architecture is particularly intriguing in the arborescent lycopsids
149 (Lepidodendrales), which speciated profusely in Carboniferous coal-swamp forests and
150 whose closest living relatives are the herbaceous quillworts (*Isoetes*). Interestingly, both
151 Lepidodendrales and *Isoetes* share a pattern of bipolar growth in which the rooting
152 system is a highly modified shoot system known as the rhizomorph²⁹. This indicates
153 that developmental studies of *Isoetes* and comparison with other living lycopsids can
154 provide insights into the development and diversification of the long-extinct arborescent
155 lycopsids³¹.

156 The examples above show that macroevolutionary studies provide invaluable
157 information on large-scale patterns of speciation and developmental evolution.
158 However, they do not provide details of the mechanisms involved in particular
159 speciation events. For that, study systems at finer evolutionary scales (closely related
160 species and populations) are required.

161

162 **The macro-microevolution transition**

163 At the macro-microevolution transition, where separation of closely related species is
164 studied, developmental repatterning can underlie the origin of reproductive barriers. It
165 also plays a key role in generating further phenotypic changes that distinguish closely
166 related species (Fig. 1). All possible types of developmental repatterning may be
167 involved in these speciation events, including changes in timing, spacial distribution,
168 quantity and type of developmental activities at each of the molecular, cellular and
169 organismal levels³². Examples of developmental repatterning between closely related
170 species (directly involved in reproductive isolation or not) include shifts in flowering
171 time³³, inflorescence architecture³⁴, nectar spur length³⁵, flower colour³⁶, petal cell
172 shape³⁷ and leaf shape³⁸. Repatterning of multiple developmental traits frequently

173 occurs associated with a speciation event. The most conspicuous examples involve
174 shifts in pollination syndromes, commonly studied through the developmental and
175 genetic comparison of closely related species with contrasting pollinators (eg in
176 *Mimulus*³⁹, *Petunia*⁴⁰, *Aquilegia*⁴¹; Fig. 1b,c; Box 1).

177 **Shifts in pollination syndrome.** Perhaps the best studied system in which evo-devo
178 research has shed light on the separation of closely related species is the Solanaceous
179 genus *Petunia*. The 20 species of *Petunia* originated in South America, and their
180 radiation is considered to have occurred within the last 3 million years⁴². Major
181 phenotypic differences between species are mostly related to the flower, and these
182 differences underpin divergent relationships with different pollinating animals⁴³.
183 Specifically, attention has focused on understanding the differences in corolla tube
184 length, stigma exsertion, anthocyanin production and UV-absorbing flavonol content
185 that distinguish bee pollinated species such as *P. integrifolia* and *P. inflata* (short tube,
186 no stigma exsertion, anthocyanin, no flavonols), moth pollinated species such as *P.*
187 *axillaris* (long tube, no stigma exsertion, no anthocyanin, flavonols) and the single
188 hummingbird pollinated species *P. exserta* (long tube, stigma exsertion, anthocyanin, no
189 flavonols) (Fig. 1b). These studies have been facilitated by the ability to cross these
190 different species and by the development of a range of genetic, genomic and transgenic
191 resources. By combining these approaches to isolate individual traits of the different
192 pollination syndromes, and by using pollinator behaviour studies, it has been possible,
193 for several of these characters, to identify both the molecular basis of trait repatterning
194 and the consequences for pollinator behaviour and reproductive isolation^{40,44-45}. These
195 combined studies in a single system have revealed novel conceptual insights into the
196 developmental shifts underpinning ecological speciation. One such insight is the
197 discovery that many of the molecular changes target transcriptional regulators of

198 developmental pathways, with the R2R3-MYB family of transcription factors being a
199 key target in *Petunia*. These proteins can be thought of as mid-level control points,
200 downstream of the essential regulation of floral organ identity but specifying the shape,
201 pattern and colour of those organs by direct activation of structural genes encoding
202 enzymes and cytoskeletal components⁴⁶. A second insight is that multiple molecular
203 evolutionary events may underpin the same phenotypic change if that change is of
204 sufficient selective advantage. Hoballah et al.⁴⁰ reported that loss of function of AN2, a
205 MYB regulator of anthocyanin production, had occurred at least five times
206 independently in wild-sampled *P. axillaris*, a white-flowered moth-pollinated species.
207 Perhaps most striking, though, has been the unexpected discovery that many of the
208 genes controlling traits involved in pollinator specificity have become linked in *Petunia*,
209 generating a multigene “speciation locus” (or “speciation island”) on chromosome II⁴⁷.
210 This is a novel feature of *Petunia* – the same genes regulating anthocyanin production,
211 UV absorption, male and female reproductive organ position and scent are distributed
212 across multiple chromosomes in other Solanaceous species. It is likely that this
213 clustering of key genes promotes linkage disequilibrium and avoids pollination
214 syndromes being disturbed by recombination that could reduce fitness.

215 ***Reproductive isolation in other ways.*** The *Petunia* system emphasizes the importance
216 of multiple trait repatterning to ensure reproductive isolation through differential
217 pollination syndromes. However, single aspects of flower development can also diverge
218 between closely related species, generating reproductive isolation more simply. One
219 classic example is the divergence of flowering time between closely related species
220 growing in the same habitat. This displacement of flowering phenology has been
221 observed in multiple systems under different conditions, and is particularly striking
222 when repeated in multiple different habitats (eg Lobo et al.⁴⁸ studying flowering time of

223 bat pollinated Bombaceae in three different habitats with different rainfall patterns).
224 Displacement of flowering can occur to minimize competition for pollinator attention
225 and interspecific hybridisation between established species, or it may occur as part of
226 the process of reproductive isolation as species diverge. Ellis et al.³³ observed
227 displacement of flowering time over a 14-week winter rainfall season for species of the
228 stone plant *Argyrodema*, and interpreted this displacement as an adaptation to isolate
229 populations that had diverged in their tolerance for different soil conditions, facilitating
230 full speciation. The processes that determine when a plant flowers are well described in
231 *Arabidopsis thaliana*, with multiple environmental and endogenous pathways
232 converging on the activity of a set of floral meristem identity genes (reviewed by Holt et
233 al.⁴⁹, Glover⁵⁰). To fully understand the molecular basis of the developmental transitions
234 in flowering time, observed in various plant radiations, multi-species studies will need
235 to be connected to the micro-evo-devo work currently exploring variation in flowering
236 time in different ecotypes of *Arabidopsis*⁵¹⁻⁵³.

237 Reproductive isolation between close relatives can also result from shifts in breeding
238 system. Shifts from outcrossing to selfing in flowering plants are commonly associated
239 with the evolution of a set of phenotypic traits known as the “selfing syndrome”:
240 smaller flowers, reduced pollen production and loss of scent and nectar production⁵⁴.

241 The developmental changes producing the selfing syndrome are being studied in the
242 sister species *Capsella grandiflora* (outcrossing, large flowers) and *C. rubella* (selfing,
243 small flowers). Reduced petal size in *C. rubella* results from a reduction in the number
244 of petal cells caused by a shortening of the cell division period. Allelic variation in the
245 intron of a general growth regulator, affecting the levels of STERILE APETALA (SAP)
246 protein in developing petals, has contributed to this change⁵⁵. Interestingly, it seems that
247 the small-petal allele of SAP was already present in the ancestral outcrossing

248 population, explaining the rapid evolutionary reduction of petal size during speciation.
249 In addition, *C. rubella* has lost a major component of floral scent present in *C.*
250 *grandiflora* (benzaldehyde) as a result of repeated inactivations of the *CNLI* gene
251 (encoding the enzyme cinnamate:coA ligase), caused by independent mutations in its
252 coding sequence⁵⁶.

253 Another example of reproductive isolation generated through an evolutionary change to
254 a developmental programme is the specialisation of plant species on pollinators with
255 particular lengths of feeding apparatus, through transitions in the length of floral tubes
256 and nectar spurs (see Box 1). This sort of change can be associated with major shifts of
257 pollinator, and therefore with other changes to flower morphology and colour, or it can
258 occur in isolation of other traits and simply select between insects of different proboscis
259 length. In a classic study of nectar spur evolution in North American species of
260 *Aquilegia*, Whittall and Hodges⁵⁷ demonstrated that the evolution of increasingly long
261 spurs is driven by speciation events involving shifts between pollinators with
262 increasingly long mouth parts (bees, hummingbirds and hawkmoths). Other studies
263 have focused on simpler transitions in spur length between closely related species of
264 orchid⁵⁸ and *Linaria*⁵⁹. Current advances in our understanding of nectar spur
265 development are allowing analysis of the molecular evolutionary processes
266 underpinning these speciation events^{35,60-61} (see Box 1).

267 ***Speciation by hybridisation and polyploidisation.*** Hybridisation and polyploidy can
268 rapidly generate reproductive isolation and therefore lead to speciation in plants⁶². In the
269 genus *Tragopogon*, for example, new allopolyploid species (*T. miscellus*, *T. mirus*) have
270 evolved in the last century in North America as a result of hybridisation between three
271 naturalised Eurasian species (Fig. 1d). Each allopolyploid has been produced multiple
272 times in independent hybridisation events, and they can also be generated synthetically,

273 providing excellent opportunities for comparative analysis. Upon allopolyploidisation,
274 genes inherited from the progenitor species can be differently expressed, silenced or
275 even lost⁶³. This may lead to developmental variation, such as that found between
276 populations of *T. miscellus*, which display long or short ligules depending on the
277 identity of the maternal and paternal parents.

278 ***Taxonomically diagnostic traits.*** While it is tempting to focus on traits directly
279 involved in reproductive isolation, phenotypic changes resulting from developmental
280 repatterning are frequently used to taxonomically delimit species, even if their
281 involvement in the initial stages of reproductive isolation is uncertain. One example is
282 evolution of leaf shape, which has been studied in the genera *Antirrhinum*³⁸ (Fig. 1a)
283 and *Solanum*⁶⁴. *Antirrhinum* comprises around 25 species, originating around 4 million
284 years ago in the Mediterranean region⁶⁵. Analysis of QTLs associated with leaf size and
285 shape following crosses between small-leaved and large-leaved species suggested that
286 the species had diverged in response to fluctuating selection regimes, consistent with a
287 radiation in a period of climate and vegetation cycles³⁸. Leaf size and shape are
288 understood as a product of the combined amount of cell division and cell expansion that
289 occurs throughout organ development, and these processes are controlled in a
290 coordinate way. Molecular evolution of these processes is often developmentally
291 constrained, causing leaves and other organs to evolve together³⁸, generating major
292 phenotypic differences that can be used in taxonomic species description as well as
293 underpinning selection in different environmental conditions. In *Solanum*, closely
294 related species display contrasting levels of complexity of compound leaves. Variation
295 in expression of the BLADE-ON-PETIOLE (BOP) transcription factor seems to explain
296 this diversity through dynamic rewiring of interactions in the gene regulatory network
297 for leaf development⁶⁴. This includes the alteration of the transcript levels of *KNOX*

298 genes, which have been recurrently recruited to generate leaf diversity during plant
299 evolution.

300 The examples in this section illustrate evolutionary developmental changes involving
301 recently diversified species, usually with reproductive barriers already in place. To
302 better understand how traits involved in speciation first evolve, developmental variation
303 can be investigated at an even finer scale, within species and populations.

304

305 **Microevolutionary scale**

306 *Genetically based intraspecific variation.* Relatively little attention has been paid to
307 microevolution in the plant evo-devo literature. Microevolutionary processes have been
308 traditionally studied from two interacting perspectives: (1) population genetics,
309 including the study of genetic variation in populations and allele frequency changes due
310 to mutation, selection, migration and drift; and (2) evolutionary ecology, which
311 investigates the biotic and abiotic interactions underlying the selective pressures that
312 lead to evolutionary change in populations. A deeper understanding of plant speciation
313 emerges from the integration of these approaches with developmental biology. The evo-
314 devo approach to microevolution (micro-evo-devo⁵) examines evolvability, the ability
315 of species and populations to produce heritable phenotypic variation, as determined by
316 genetic architecture and developmental constraints^{6,66}. In this way, it provides insight
317 into the processes that supply the raw material for adaptation, evolution and
318 speciation^{5,67}. This generally involves the study of developmental variation across
319 populations of the same species and within populations, particularly those
320 polymorphisms that may underlie local adaptation, divergence between populations and,
321 potentially, the establishment of reproductive barriers (Fig. 2).

322 A fertile field for microevolutionary research in plants is the study of flower colour
323 polymorphisms. Flower pigmentation is involved in pollinator specialisation^{23,68}, and its
324 molecular and developmental basis has been well studied^{22,24}. In addition, intraspecific
325 colour polymorphisms are relatively common in nature, and they frequently involve few
326 genetic changes. A link between intraspecific flower colour variation and incipient
327 diversification has been demonstrated in the sticky monkey-flower (*Mimulus*
328 *aurantiacus*). A number of studies⁶⁹⁻⁷¹ have addressed the genetic basis of flower colour
329 variation, population genetics, pollinator interactions and isolating barriers in two
330 closely related morphs of *M. aurantiacus* distributed in southwestern California: a red-
331 flowered ecotype preferentially pollinated by hummingbirds and a yellow-flowered one
332 preferred by hawkmoths (Fig. 2a). This multi-disciplinary approach has revealed
333 incipient ecological speciation in the face of gene flow, primarily resulting from
334 pollinator preferences causing divergent selection on an R2R3-MYB transcription factor
335 (*MaMyb2*) involved in the regulation of the anthocyanin biosynthetic pathway⁷¹. A *cis*-
336 regulatory change in *MaMyb2* is responsible for the colour change underlying incipient
337 pre-mating isolation between ecotypes. This example reveals microevolutionary
338 mechanisms by which traits involved in pollination syndrome shifts first evolve.

339 Another trait relevant to speciation that is amenable to population-level research is floral
340 symmetry. Floral zygomorphy is known to have evolved in several genera of
341 Brassicaceae in correlation with differences in expression of *CYC2* during corolla
342 development⁷². To understand how zygomorphy has evolved at a microevolutionary
343 scale, an ideal system is provided by *Erysimum mediohispanicum*, a member of the
344 Brassicaceae displaying heritable intraspecific variation in floral symmetry, from
345 actinomorphic to zygomorphic⁷³ (Fig. 2b). Evolutionary ecological approaches show
346 that plants bearing zygomorphic flowers have the highest fitness, and that strong

347 selection on corolla shape is exerted by pollinators⁷⁴. By analyzing the developmental
348 genetic basis of floral symmetry variation in *E. mediohispanicum*, the
349 microevolutionary process by which zygomorphy evolves would be more fully
350 understood, and this would in turn enhance our understanding of macroevolutionary
351 patterns in Brassicaceae.

352 Many other traits potentially involved in speciation are being investigated using
353 polymorphic target species, including the following examples: flowering time
354 differentiation between locally adapted populations in *Arabidopsis thaliana*⁵¹;
355 continuous variation in pollination by sexual deception in the South African beetle daisy
356 (*Gorteria diffusa*; Fig. 2c)⁷⁵; the recurrent parallel divergence of morphologically
357 distinct ecotypes adapted to contrasting habitats in the Australian groundsel *Senecio*
358 *lautus*⁷⁶; and adaptive variation in the production of leaf trichomes, involved in
359 resistance to herbivory, in *Arabidopsis lyrata*⁷⁷. There is the exciting potential for the
360 comparative microevolutionary study of similar traits in distant lineages to provide
361 metamodels linking the macro- and microevolutionary scales.

362 **Beyond genetic variation.** While intraspecific phenotypic variation discussed thus far is
363 considered to be the result of genetic changes, recent research has highlighted a
364 potential role in speciation for other components of variation. Phenotypic plasticity, the
365 capacity of a genotype to produce alternative phenotypes in response to environmental
366 variation, has been suggested as a facilitator of adaptive divergence and speciation⁷⁸⁻⁷⁹.
367 Intraspecific phenotypic differences initially generated by plasticity may be fixed in
368 different populations by natural selection in the process of genetic assimilation, and can
369 then contribute to potentially rapid genetic divergence, reproductive isolation and
370 eventually speciation⁷⁸. As a result, the developmental mechanisms responsible for
371 plasticity may parallel those underlying interspecific diversity. For example,

372 heterophylly in the North American lake cress (*Rorippa aquatica*), involving
373 morphological differences between leaves developing under submerged and terrestrial
374 conditions, is the result of environmentally induced changes in the expression of
375 *KNOX1* genes, which are also implicated in the diversification of leaf shape across
376 species of the same family⁸⁰.

377 Related to phenotypic plasticity, there is speculation that heritable epigenetic variation,
378 shaped by the environment and natural selection, might also aid evolutionary change
379 and speciation⁸¹⁻⁸³. Epigenetic diversity, triggered by environmental changes, may
380 enable genetically depauperate populations to quickly adapt until genetic assimilation
381 fixes phenotypic differences. Interestingly, one of the first naturally occurring
382 morphological mutants to be genetically characterized, the peloric mutant of *Linaria*
383 *vulgaris* (showing radially symmetrical flowers instead of the zygomorphic flowers that
384 are characteristic of *Linaria*), was found to be an epimutant resulting from extensive
385 methylation of the *CYC* gene⁸⁴. Although the evolutionary significance of this particular
386 mutant is probably limited given its compromised reproductive success, evidence has
387 since been found of heritable intraspecific epigenetic variation correlated with
388 phenotypic differences and potentially subject to natural selection⁸⁵. For example, in the
389 yellow monkeyflower (*Mimulus guttatus*), epigenetically inherited variation in trichome
390 density is induced by herbivore damage, and is correlated with differential regulation of
391 a *MYB MIXTA-like* transcription factor⁸⁶. The emerging field of population epigenetics,
392 combined with ecological and developmental approaches, can provide insights into this
393 still largely hypothetical link between epigenetics and speciation.

394

395 **Concluding Remarks**

396 The processes of speciation have puzzled evolutionary biologists for over 150 years.
397 Many models to explain how new species emerge have been proposed and many
398 systems developed in which to test those models. It is clear that speciation events result
399 from a combination of multiple molecular, environmental and stochastic factors.
400 However, the recent input of evolutionary developmental biology into this field has
401 generated new insights. It has allowed both the crystallisation of novel concepts
402 surrounding speciation processes and the revisiting of old questions (Box 2). We
403 conclude that a strong interaction between developmental biology and evolutionary
404 biology (including phylogenetics, population genetics, evolutionary ecology,
405 paleontology; Box 3) is crucial to retain momentum in the drive to gain a deeper
406 understanding of plant speciation.

407

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411

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614 **Figures**

615

616 **Figure 1 | Model systems for the evolutionary developmental study of plant**
617 **speciation. a**, *Antirrhinum* spp.: variation in flower and leaf morphology in a recent
618 radiation, represented by *A. majus* (left), *A. braun-blanquetii* (centre) and *A. charidemi*
619 (right). **b**, *Petunia* spp.: transitions in flower tube length, stigma exertion and colour
620 associated with pollinator shifts, represented by *P. inflata* (bee-pollinated, left), *P.*
621 *exserta* (hummingbird-pollinated, centre) and *P. axillaris* (moth-pollinated, right). **c**,
622 *Mimulus* spp.: differences in floral morphology between two sister species with
623 contrasting pollination syndromes, *M. lewisii* (bee-pollinated, left) and *M. cardinalis*
624 (hummingbird-pollinated, right). **d**, *Tragopogon* spp.: capitula of the allopolyploid
625 hybrid *T. mirus* (centre) and its parent species *T. dubius* (left) and *T. porrifolius* (right).
626 Photos by A. Hudson (a), H. Sheehan (b), H.D. Bradshaw (c), E. Mavrodiev (d, left and
627 centre) and A.N. Doust (d, right).

628

629 **Figure 2 | Species showing intraspecific variation in developmental traits relevant**
630 **to plant speciation. a**, *Mimulus aurantiacus*: floral colour variation associated with
631 pollinator preferences between a red-flowered ecotype (preferred by hummingbirds,
632 left) and a yellow-flowered ecotype (preferred by hawkmoths, right). **b**, *Erysimum*
633 *mediohispanicum*: variation in floral symmetry correlated with fitness differences,
634 including radial (left), dissymmetric (centre) and zygomorphic (right) flowers. **c**,
635 *Gorteria diffusa*: variation in presence of petal spots and degree of sexual deception
636 between three morphotypes (from left to right: Steinkopf, Cal and Buffels). Photos by
637 M.A. Streisfield (a), J.M. Gómez (b) and G. Mellers (c).

638

639

640 **Box 1 | Nectar spurs and speciation: from macro- to microevolution.**

641 Floral nectar spurs constitute one of the best examples of a trait involved in plant
642 speciation that is being studied at multiple evolutionary scales, integrating evolutionary,
643 developmental and ecological perspectives. These spurs are tubular outgrowths of floral
644 organs usually containing a nectar reward for pollinators, and they have evolved
645 multiple times during the diversification of flowering plants, within families as distantly
646 related as Ranunculaceae, Orchidaceae, Violaceae and Plantaginaceae⁸⁷. Phylogenetic
647 comparative analyses suggest bursts of diversification associated with clades with
648 nectar spurs, leading to the consideration of this trait as a “key innovation” promoting
649 speciation through pollinator specialisation^{14,87}.

650 At the *macroevolutionary scale*, the comparative study of nectar spurs in different
651 families allows us to test the degree to which similar ontogenetic and genetic
652 mechanisms have been recurrently recruited to produce a morphologically and
653 functionally convergent trait. Thus far, ontogenetic mechanisms producing nectar spurs
654 seem to be broadly similar across families, with an initial phase of cell division
655 followed by a phase of cell elongation^{35,60,88}. However, recent evidence suggests a
656 diversity of molecular mechanisms recruited to achieve this, with *KNOX* and *TCP4*
657 genes respectively proposed as regulators of spur development in toadflaxes (*Linaria*,
658 Plantaginaceae)⁶⁰ and columbines (*Aquilegia*, Ranunculaceae)⁶¹.

659 At the *macro-microevolution transition*, recent speciation potentially driven by nectar
660 spurs has been studied mainly in the North American clade of the genus *Aquilegia*. In
661 this lineage, changes in spur length are associated with shifts in the main pollinators
662 (bees, hummingbirds or hawkmoths)⁵⁷, and spur length differences contribute to the
663 reproductive isolation between co-occurring species⁸⁹. Ontogenetically, variation in spur
664 length across species is the result of changes in the duration of the phase of cell

665 elongation during spur development³⁵. It remains to be determined whether this
666 heterochronic mechanism of spur length evolution also characterises other unrelated
667 spurred lineages.

668 The *microevolutionary scale* provides the best opportunities to test evolutionary models
669 of spur length change, such as the coevolutionary race and pollinator shift hypotheses⁵⁷,
670 and the degree to which spur evolution contributes to incipient divergence. In
671 Scandinavian populations of the orchid *Platanthera bifolia*, geographic variation in spur
672 length is correlated with the proboscis length of distinct local pollinators, suggesting
673 that intraspecific changes in spur length are driven by pollinator shifts⁹⁰. The
674 developmental mechanisms behind these changes, as well as their potential contribution
675 to incipient divergence and eventual speciation, remain to be studied.

676

677 **[Box 1 Figure] Model systems to study the evolution of nectar spurs.** Ordinal-level
678 phylogeny of flowering plants, with red branches indicating orders in which nectar
679 spurs have evolved⁸⁷. Three phylogenetically disparate genera in which evolution of
680 nectar spurs is being investigated at different scales are shown: (a) *Aquilegia*: species
681 with different pollination syndromes display contrasting spur lengths, as exemplified by
682 *A. sibirica* (c. 10 mm, bee-pollinated, top), *A. formosa* (c. 20 mm, hummingbird-
683 pollinated, centre) and *A. chrysantha* (c. 70 mm, hawkmoth-pollinated, bottom). (b)
684 *Linaria*: *L. salzmannii* (c. 13 mm, left) and *L. clementei* (c. 3 mm, right) are two closely
685 related species with nearly identical floral morphology but contrasting spur lengths. (c)
686 *Platanthera*: intraspecific variation in spur length in *P. bifolia* (c. 15-40 mm) correlates
687 with the proboscis length of local pollinators. Nectar spurs are indicated by arrow heads
688 in all photos. Photos by E.S. Ballerini (a), M. Fernández-Mazuecos (b) and J. Quiles (c).
689

690 **Box 2 | Hopeful monsters?**

691 The evolutionary relevance of large-effect mutations in evolution and speciation is at
692 the centre of a long-standing debate in evolutionary biology¹⁰. While widely accepted
693 evolutionary models regard gradual change as the most likely mode of evolution, it has
694 been frequently argued by developmental biologists that homeotic mutations, changing
695 the identity of whole organs, may have played a role in some major evolutionary
696 transitions²⁻³. For example, evolutionary changes in floral organ identity have been
697 hypothesised to be the result of homeotic mutations involving changes in the expression
698 domains of genes in the ABC model of flower development⁹¹. While this hypothesis is
699 intriguing, systems in which the feasibility of such changes can be studied at the
700 microevolutionary scale are required to test it. The *Stamenoid petals (Spe)* mutant of the
701 shepherd's purse (*Capsella bursa-pastoris*) has been proposed as a suitable model for
702 this⁹². It is a naturally occurring floral homeotic mutant in which petals have been
703 transformed into stamens. Unlike other known homeotic mutants, it forms stable
704 populations in the wild, mixed with wild-type plants. The mutation has been shown to
705 be heritable and involves a single locus, hypothesized to be a class C floral identity
706 gene⁹³. Both morphs seem to have similar fitness, and a degree of genetic differentiation
707 and reproductive isolation between them has been detected in a German locality,
708 suggesting incipient speciation⁹⁴⁻⁹⁵. Even if considered a rarity, this system nicely
709 bridges microevolutionary processes and macroevolutionary outcomes, and hints at the
710 feasibility of saltational changes giving rise to “hopeful monsters” of potential long-
711 term evolutionary relevance⁹⁶.

712

713 **[Box 2 Figure] A hypothetical “hopeful monster”.** Flowers of wild-type *Capsella*
714 *bursa-pastoris* (left) and the naturally occurring *Spe* mutant of the same species (right).
715 Photos by G. Theißen.
716
717

718 **Box 3 | The need for a robust phylogenetic context.**

719 The role of phylogenetics in evolutionary developmental biology has been highlighted
720 since the origins of evo-devo⁹⁷, and it is particularly crucial when the focus is on
721 speciation. Indeed, phylogenetic relationships have to be known if the sequence and
722 direction of developmental changes in the course of speciation are to be understood⁹⁸,
723 including, for example, the detection of instances of parallelism that may result from
724 developmental biases. However, integration of phylogenetic and developmental data is
725 often lacking, and the use of new analytic tools to achieve it is desirable⁹⁹⁻¹⁰⁰. In
726 addition, speciation studies frequently involve recently diverged species or populations
727 whose phylogenetic relationships cannot be easily resolved using conventional
728 phylogenetic approaches. To that end, high throughput sequencing methods capable of
729 providing genome-wide markers are required. In their study of flower colour divergence
730 during incipient diversification in the *Mimulus aurantiacus* complex, for example,
731 Stankowski & Streisfeld⁷⁰ provide a good example of the use of a robust phylogenetic
732 framework, developed using RAD-Seq markers, to reconstruct evolutionary
733 developmental changes. According to phylogenetic analyses, red flowers have been
734 acquired in two independent lineages with yellow flowered ancestors. In both cases, the
735 red pigmentation is the result of a *cis*-regulatory mutation in the gene *MaMyb2*.
736 Interestingly, population genetic analyses suggest that a single red allele may have
737 evolved and subsequently been transferred between the two red-flowered morphs by
738 introgression.