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1 **The *flexible stem hypothesis*: evidence from genetic data**

2

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11

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21 **Abstract: Phenotypic plasticity, the ability of a given genotype to produce**  
22 **different phenotypes in response to distinct environmental conditions, is**  
23 **widely observed in the wild. It is believed to facilitate evolution and, under**  
24 **the “flexible stem hypothesis”, it is thought that an ancestral plastic species**  
25 **can be at the origin of sister lineages with divergent phenotypes fixed by**  
26 **genetic assimilation of alternative morphs. We review here the genetic**  
27 **mechanisms underlying such phenomenon. We show several examples in**  
28 **which the same gene shows transcriptional plasticity in response to**  
29 **environmental factors and divergence of expression within or between**  
30 **species. Thus, the same gene is involved both in the plasticity of a trait and**  
31 **in the evolution of that trait. In a few cases, it can be traced down to *cis-***  
32 **regulatory variation in this gene and, in one case, in the very same**  
33 **regulatory sequence whose activity is modulated by the environment.**  
34 **These data are compatible with the “flexible stem hypothesis” and also**  
35 **suggest that the evolution of the plasticity of a trait and the evolution of the**  
36 **trait are not completely uncoupled as they often involve the same *locus*.**  
37 **Furthermore, the “flexible stem hypothesis” implies that it is possible to**  
38 **canalize initially plastic phenotypes. Several studies have shown that it was**  
39 **possible through modification of chromatin regulation or hormonal**  
40 **signalling/response. Further studies of phenotypic plasticity in an**  
41 **evolutionary framework are needed to see how much the findings**  
42 **described in this review can be generalized.**

43

44 **Key words: Phenotypic plasticity, Evolution, Gene expression, Review**

45

46 In developmental biology, the environment has long been thought to fulfil  
47 essentially a permissive role, simply providing the required conditions for  
48 normal development to occur. Thus, most developmental biology laboratories  
49 still use standardized environmental conditions and focus on genetic factors to  
50 dissect developmental mechanisms. However, in many cases the environment  
51 has an instructive role, directing the development to particular alternative path  
52 (Gilbert 2001). Phenotypic plasticity is a major concept which describes "*the*  
53 *property of a given genotype to produce different phenotypes in response to*  
54 *distinct environmental conditions*" (Pigliucci 2001). It is widely observed in the  
55 wild, and this concept was formulated by Bradshaw in the sixties (Bradshaw  
56 1965). Phenotypic plasticity can be an adaptation to fluctuating environments  
57 such as seasonal variations, presence/absence of predators, or population  
58 density. It can also be non adaptive and Schmalhausen used the term "*morphose*"  
59 to describe aberrant phenotypes induced by extreme environmental conditions  
60 (Schmalhausen 1949). A major tool to illustrate phenotypic plasticity is the  
61 reaction norm, first drawn by Woltereck in 1909 (Woltereck 1909), which  
62 represents the value of a quantitative phenotype produced by a given genotype  
63 in function of an environmental parameter. Note that "*genotype*" and "*phenotype*"  
64 were defined only shortly after by Johannsen (1911) who reinterpreted the  
65 reaction norms of Woltereck (Johannsen 1911). Indeed, Woltereck observed  
66 that different pure lines could produce different morphologies in a given  
67 environment but the same morphology in another environment. He interpreted  
68 this as inconsistent with the existence of constant differences between the  
69 genotypes. Because he did not distinguish between genotypes and phenotypes,

70 he thought that the different pure lines had the same genotype in a given  
71 environment (when they had the same phenotype) and different genotypes in  
72 another environment (when they had different phenotypes). When phenotypic  
73 plasticity leads to discrete morphs, these morphs are called polyphenisms.  
74 Phenotypic plasticity can generate, within a single species, morphological  
75 differences of a magnitude usually observed between distantly related species.  
76 As selection operates on phenotypes, this direct input of the environment on the  
77 expression of the genotype must be taken into account to understand  
78 evolutionary processes. This was clearly formulated by Schmalhausen (1949)  
79 (Schmalhausen 1949): "*Darwin has previously noted the fundamental importance*  
80 *of inherited variability (mutations) for the evolution of organisms. However,*  
81 *mutations have different expressions under diverse conditions. If natural selection*  
82 *is the basic factor in evolution, then the concrete expression of individual*  
83 *characteristics under given environmental conditions is of decisive importance in*  
84 *this process*». Thus, the role of phenotypic plasticity in evolution has been  
85 actively discussed (Pfennig et al. 2010; Moczek et al. 2011; Laland et al. 2014,  
86 2015; Schneider and Meyer 2017). The issue is complex and certain empirical  
87 results suggest that it is important to distinguish between adaptive and non-  
88 adaptive plasticity as they may have opposite effects on genetic divergence  
89 (Ghalambor et al. 2015).

90 The idea that an ancestral plastic species could have been at the origin of  
91 divergent lineages with pronounced phenotypic differences has been proposed  
92 by Mary-Jane West-Eberhard as the "*flexible stem hypothesis*" (West-Eberhard  
93 2003). It is intimately linked to the process of genetic assimilation discovered by  
94 Conrad Waddington more than 50 years ago (Waddington 1952, 1956, 1959). In

95 his experiments on *Drosophila*, Waddington showed that a phenotype initially  
96 induced by the environment can be fixed by selection and become constitutively  
97 expressed even in the absence of the initial environmental factor. Most of these  
98 experiments were based on developmental defects induced by stressors such as  
99 heatshock (crossveinless phenotypes on wings) (Waddington 1952) or ether  
100 vapour (*bithorax* phenocopies) (Waddington 1956). However, they could be  
101 generalized to adaptive phenotypes and had therefore major evolutionary  
102 implications. Indeed, in another Waddington experiment, the morphological  
103 change induced by the environment, *i.e.* size increase of the anal papilla of larvae  
104 in response to high salt concentration, was likely adaptive and was also  
105 genetically assimilated (Waddington 1959). Importantly, genetic assimilation is a  
106 genuine evolutionary process based on selection of genetic variation existing in  
107 the population submitted to environmental changes. Indeed, Waddington  
108 experiments were later repeated by Bateman on outbred or isogenized stocks  
109 and it was shown that genetic assimilation did not happen in the absence of  
110 genetic variation in the population (Dworkin 2005). This genetic variation is  
111 cryptic in the original environment in which it accumulates as it has no  
112 phenotypic consequences. This genetic variation translates into phenotypic  
113 variation in the new environment, which allows genetic assimilation by  
114 selection. Thus, a lineage characterized by a constitutively expressed morph  
115 could evolve from a plastic ancestor by genetic assimilation of a phenotype  
116 initially induced by environmental change.

117 This has lead Mary Jane West-Eberhard to propose that genes act more as  
118 followers than as leaders during evolution (West-Eberhard 2005). Although very  
119 stimulating, this idea cannot be generalized to the evolution of all traits. Indeed,

120 in many examples, genetic polymorphisms have played a major role in the origin  
121 of a phenotype later fixed during evolution (Schwander and Leimar 2011). For  
122 example, a particular allele of the pigmentation gene *tan* leading to lighter  
123 pigmentation is segregating in *Drosophila americana* and has been fixed in its  
124 sister species *Drosophila novamexicana* which has a derived pale phenotype  
125 (Wittkopp et al. 2009). However, although the “*plasticity first hypothesis*” does  
126 not apply to all traits, it is worth investigating it empirically. The flexible stem  
127 model of adaptive radiation predicts that the pattern of phenotypic divergence  
128 between derived lineages should mirror the pattern of developmental plasticity  
129 in their common ancestor (Wund et al. 2008). Several studies have provided  
130 empirical morphological data strongly suggesting that it could occur in nature  
131 (Wund et al. 2008). For example, the pharyngeal jaws of midas cichlids show a  
132 morphological plasticity in response to the hardness of the diet which parallels  
133 the morphological diversity observed between specialised species (Muschick et  
134 al. 2011). Similarly, stress-induced changes in mandible shape in *Sorex* shrews  
135 correspond to traits showing divergence between species (Badyaev and  
136 Foresman 2000).

137 What we would like to review and discuss in this article is the underlying genetic  
138 bases of the flexible stem hypothesis. Indeed, under this hypothesis, it is  
139 expected that the genetic mechanisms that generate the plastic phenotypes  
140 should be the same as those that generate the phenotypic divergence. The idea  
141 that phenotypic plasticity is under genetic control was initially developed by  
142 Bradshaw (Bradshaw 1965) and a recurrent question was the existence and the  
143 nature of “*plasticity genes*” (Via et al. 1995). However, Via considered that  
144 modifications of reaction norms was an indirect consequence of the selection on

145 the mean phenotype in each separate environment and that no plasticity genes  
146 were required (Via 1993). Scheiner, Schlichting and Pigliucci objected to Via that  
147 the plasticity of a trait could evolve independently of the mean of the trait  
148 because the plasticity and the mean of the trait are under the control of different  
149 genes (Scheiner 1993; Schlichting and Pigliucci 1993). This debate settled down  
150 in the mid nineties in favour of the existence of plasticity genes. Two kinds of  
151 genetic mechanisms involved in phenotypic plasticity were proposed (Via et al.  
152 1995). The first one, "*allelic sensitivity*", refers to alleles expressed differently in  
153 distinct environment. This could correspond to a single gene showing  
154 transcriptional plasticity or to an enzyme active in only some environmental  
155 conditions for example. The second genetic mechanism, "*gene regulation*",  
156 corresponds to a regulatory switch under environmental control, which would  
157 lead to the activation of a panel of target genes depending on the environment.  
158 Obviously, the two mechanisms may blur as the regulatory *locus* influences the  
159 amount of products of its targets, thus mediating allelic sensitivity (Via et al.  
160 1995). Clearly, a great deal of information could be gained by genetic dissection  
161 of phenotypic plasticity in a variety of organisms.

162 A few empirical studies have identified genes involved in the phenotypic  
163 plasticity of particular traits and in several cases, data are available regarding  
164 genes involved in the variation within or between species of the traits of interest.  
165 Thus, it is possible to analyse the genetic changes that underlie genetic  
166 assimilation of initially plastic traits. Furthermore, several studies have dissected  
167 the mechanisms involved in phenotypic plasticity and environmental  
168 canalization. The typical questions we would like to address are the following:  
169 What are the relationships between genes involved in phenotypic plasticity of a



170 trait and genes involved in the evolution of this trait? What kind of mutations are  
171 selected during the evolution of these traits? Are they regulatory or coding  
172 mutations? Do they target *cis*-regulatory sequences of a modulated gene or *trans*-  
173 acting factors? How is it possible to make independent of the environment an  
174 initially plastic trait? We present here several examples of phenotypic plasticity,  
175 chosen in animals or plants, whose genetic bases have been addressed through  
176 candidate gene approach or global transcriptome analyses. Most of these  
177 analyses are performed in an evolutionary context (either artificial or natural) or  
178 can be complemented by other studies on the evolution of the particular traits of  
179 interest in closely related species. Furthermore, we describe studies dissecting  
180 the mechanisms involved in phenotypic plasticity and environmental  
181 canalisation. Modulation of such mechanisms must be involved in the  
182 environmental canalization of initially plastic traits involved in the flexible stem  
183 hypothesis.

184

#### 185 **Ether-induced *Ultrabithorax* phenocopies in *Drosophila***

186 Treatment of *Drosophila* embryos with ether vapor leads to phenocopies of the  
187 *bithorax* mutant in adults (Waddington 1956). This corresponds to partial  
188 homeotic transformation of the third thoracic segment into the second thoracic  
189 segment. In *Drosophila*, like in all dipterans, the dorsal appendages of the third  
190 thoracic segments are not wings but little round structures named halteres. In  
191 *bithorax* phenocopies, halteres are partially transformed into wings and the  
192 postnotum is partially transformed into an additional mesonotum (Figure 1).  
193 Waddington showed that this ether-induced phenotype can be genetically  
194 assimilated (Waddington 1956). By selection after ether treatment at each

195 generation, he could progressively increase the proportion of individual showing  
196 the *bithorax* phenocopy. At the eighth generation, this phenotype was observed  
197 in some individuals even in the absence of ether treatment (Figure 1). The  
198 *bithorax* phenotype was therefore genetically assimilated. Waddington  
199 experiments were repeated by Gibson and Hogness (Gibson and Hogness 1996).  
200 They analysed the expression of the homeotic gene *Ultrabithorax (Ubx)* which is  
201 expressed in haltere but not wing imaginal disc and is responsible for the  
202 different identities of these organs. They showed that ether-induced *bithorax*  
203 phenocopies are caused by clonal loss of expression of *Ubx* in the haltere  
204 imaginal disc. Furthermore, using polymorphism in *Ubx*, they could show that  
205 the frequency of particular alleles of *Ubx* increased during genetic assimilation of  
206 the *bithorax* phenotype. The *Ubx* polymorphism was not the only source of  
207 genetic variation affecting sensitivity to ether, but it had a major effect. Thus, *Ubx*  
208 expression is sensitive to ether and during genetic assimilation of this  
209 phenotype, artificial selection targeted the *Ubx* locus itself.

210

### 211 **Transcriptional response to osmotic challenge in killifish:**

212 The Atlantic killifish (*Fundulus heteroclitus*) can be found along a steep salinity  
213 gradient in Atlantic coast estuaries and can quickly adjust to extremes in  
214 environmental salinity (Figure 2). Thus, this species is able to occupy an  
215 extremely wide osmotic niche. Hypo-osmotic challenge induces a change of  
216 cellular morphology of the apical surface of the gill epithelium (Whitehead et al.  
217 2011) (Figure 2). In sea water, the apical surface of the gill epithelium is made of  
218 pavement cells interspersed with mitochondrion-rich cells possessing apical  
219 crypt with small surface area (Whitehead et al. 2011). Exposure to fresh water

220 leads to enlargement of the apical surface of mitochondrion-rich cells. This  
221 modification occurs within a few days, but its speed and the precise morphology  
222 of the gill epithelium differs between populations originating from different  
223 point of the salinity gradient. Analysis of the transcriptome of gills in fish  
224 submitted to hypo-osmotic challenge allowed to identify sets of genes whose  
225 expression was modulated by salinity (Whitehead et al. 2011). It was found that  
226 genes showing transcriptional plasticity during osmotic challenge are more  
227 likely to show divergence of expression between populations living in different  
228 environment (Whitehead et al. 2011, 2012). Indeed, many of the genes  
229 modulated during hypo-osmotic challenge show adaptive divergence in  
230 expression in populations living exclusively in freshwater habitat. Among these  
231 genes, many have a well known role in osmotic regulation, for example  
232 Aquaporin 3 (AQP3), the sodium/potassium transporting ATPase ATP1A1 or  
233 versican (VCAN) (Whitehead et al. 2012).

234

### 235 **Plasticity of wing eyespots in the butterfly *Bicyclus anynana***

236 The African satyrine butterfly *Bicyclus anynana* shows seasonal polyphenisms. In  
237 the wet season morph, conspicuous eyespots made of concentric rings of  
238 differently pigmented scales are observed on the ventral wings. In the dry season  
239 morph, the ventral eyespots are absent. Only a reduced version of the white spot  
240 in the centre of the eyespot is visible. The wet and dry season morphs can be  
241 produced in the laboratory by rearing the larvae at 27°C or 17°C respectively  
242 (Brakefield et al. 1996) (Figure 3).

243 Many developmental regulatory genes are expressed in developing eyespots of  
244 *Bicyclus anynana*, among which *Distal-less (Dll)* which is expressed in the future

245 centre of the eyespot (Brakefield et al. 1996; Shirai et al. 2012). This is not  
246 simply a correlation as it was recently shown by gain and loss of function  
247 experiments using transgenic *Bicyclus anynana* that *Dll* is involved in eyespot  
248 formation (Monteiro et al. 2013). Indeed, overexpression of *Dll* increases eyespot  
249 size and induces ectopic eyespots. Conversely, decreasing *Dll* expression reduces  
250 eyespot size. Furthermore, the expression of *Dll* in presumptive eyespots is  
251 modulated by temperature: it is stronger and wider in pupae grown at 27°C that  
252 will produce the wet season morph (Brakefield et al. 1996). Thus, *Dll* is one of  
253 the genes involved in eyespot plasticity.

254 It is possible to fix alternative morphs by artificial selection performed at  
255 intermediate temperature. This lead to a LOW line and a HIGH line showing  
256 almost non plastic dry season and wet season phenotypes, respectively  
257 (Brakefield et al. 1996) (Figure 3). Thus, the wet or dry season morphs can be  
258 genetically assimilated and become virtually independent of the rearing  
259 temperature. This correlates with a modification of *Dll* expression, which is  
260 stronger and wider in pupae of the HIGH line than in pupae of the LOW line  
261 grown in the same environmental conditions. Thus, genetic assimilation of the  
262 alternative seasonal morphs has involved modulation of *Dll* expression. Whether  
263 this genetic assimilation was caused by selection at the *Dll* locus itself (*cis*  
264 variation) or by *trans*-acting factors was not tested in this experiment. However,  
265 another selection experiment targeting the size of dorsal eyespots (which  
266 however are not plastic), showed that natural genetic variation in *Dll* was linked  
267 to eyespot size variation in *Bicyclus anynana* (Beldade et al. 2002). Thus,  
268 variation at the *Dll* locus with significant impact on eyespot patterning exists in  
269 *Bicyclus anynana*.

270

271 **Polyphenism of cuticular teeth in diplogastrid nematods**

272 The diplogastrid nematod *Pristionchus pacificus* displays two alternative tooth  
273 morphs, stenostomatous (St) or eurystomatous (Eu), that are produced after an  
274 irreversible decision during larval development (Bento et al. 2010) (Figure 4).  
275 Eu morph is adapted to predation on other nematods, whereas St morph is  
276 adapted to bacterial diet. The ratio of individuals with St or Eu teeth varies  
277 between strains but is also influenced by environmental factors such as  
278 crowding and starvation (Bento et al. 2010; Ragsdale et al. 2013). The high  
279 expression of the gene *eud-1*, encoding a sulfatase, is necessary and sufficient to  
280 produce the Eu phenotype in *Pristionchus pacificus* (Ragsdale et al. 2013). Loss of  
281 function mutations of *eud-1* are dominant and lead to the St phenotype.  
282 Difference of the Eu/St ratio between wild isolates of *Pristionchus pacificus*  
283 correlates with difference in *eud-1* expression. Furthermore, *eud-1* over-  
284 expression of is sufficient to transform high St strains into Eu ones. The role of  
285 *eud-1* in tooth dimorphism extends to the other diplogastrid species *Pristionchus*  
286 *expectatus* (Ragsdale et al. 2013). Indeed, through hybrid crosses between  
287 various *P. pacificus* lines and a *P. expectatus* St line, it was shown that the St  
288 phenotype of the *P. expectatus* line was caused by a low expression of *eud-1*. As  
289 transformation of *P. pacificus eud-1* mutant with the *eud-1* allele of the *P.*  
290 *expectatus* St line leads to a Eu phenotype, the *eud-1* allele of *P. expectatus* is  
291 functional and differences of *eud-1* expression between *P. pacificus* and *P.*  
292 *expectatus* are caused by differences in *trans*-acting factors. Thus, divergence in  
293 the ratio of tooth morphs might have involved several components of the *eud-1*  
294 network.

295 It is thought that the stem species in diplogastrid nematods was polyphenic  
296 (Kiontke and Fitch 2010). Many descendant species such as *Pristionchus pacificus*  
297 have kept both morphs, but others have become exclusively Eu or St.  
298 Interestingly, recent morphometric analysis of diplogastrid mouth structures in  
299 a more global evolutionary framework revealed a rapid process of diversification  
300 associated with developmental plasticity (Susoy et al. 2015): The gain of  
301 dimorphic mouth structures was associated with an increase rate of evolution  
302 and the appearance of numerous new species with diversified mouth parts.

303

#### 304 **Modulation of leaf margin dissection by temperature in *Capsella***

305 The two brassicaceae sister species *Capsella rubella* and *Capsella grandiflora*  
306 show differences in leaf margin dissection (Sicard et al. 2014). Leaf margin  
307 dissection is more pronounced in *Capsella rubella* and is increased at low  
308 temperature (16°C versus 22°C). It was shown that the major locus responsible  
309 for the difference in leaf margin dissection between *C. rubella* and *C. grandiflora*  
310 is the homeobox gene *RCO-A* which is expressed in sinuses between growing  
311 lobes of developing leaves in *C. rubella* (Sicard et al. 2014). This difference of  
312 expression level is caused by insertions in the *C. rubella* *RCO-A* promoter. The  
313 effect of the *RCO-A* region on leaf margin dissection is visible in nearly isogenic  
314 lines differing only in the region of *RCO-A*, *NILgg* (carrying the *RCO-A* allele of *C.*  
315 *grandiflora*) and *NILrr* (carrying the *RCO-A* allele of *C. rubella*) (Figure 5). In  
316 addition, *RCO-A* expression is strongly modulated by temperature. Expression of  
317 the *C. grandiflora* *RCO-A* allele is extremely weak at 22°C and the leaves show no  
318 dissection. This allele is up-regulated more than 150 folds at 16°C compared to  
319 22°C, which leads to leaf margin dissection. The *C. rubella* *RCO-A* allele is more

320 expressed than the *C. grandiflora* one at 22°C and is upregulated less than 5 folds  
321 between 22°C and 16°C. Consequently, it leads to leaf margin dissection at both  
322 temperatures in *C. rubella*.

323 In another brassicae species, *Arabidopsis thaliana*, the *RCO-A* locus is deleted and  
324 there is no leaf margin dissection. Insertion of transgenes carrying *Capsella*  
325 *rubella* *RCO-A* gene in *Arabidopsis thaliana* is sufficient to induce leaf margin  
326 dissection (Sicard et al. 2014).

327 Thus, the same *locus* is directly involved in phenotypic plasticity and species  
328 divergence regarding leaf margin dissection. However, as the activity of the  
329 regulatory sequences of *RCO-A* responsible for the difference of expression  
330 between the two *Capsella* species was not studied with a reporter gene in  
331 transgenic lines grown at different temperatures, it is not known whether they  
332 also mediate the effect of the environment.

333

### 334 **Thermal plasticity of abdominal pigmentation in *Drosophila melanogaster*** 335 **females**

336 Cold induces melanism in many insects (Gibert et al. 2000; Michie et al. 2010;  
337 Fedorka et al. 2013). This is thought to be beneficial as dark pigmentation  
338 increases body temperature. However, pigmentation is highly pleiotropic and is  
339 also correlated to many other traits such as longevity, fecundity or immunity  
340 (Wittkopp and Beldade 2009; Fedorka et al. 2013; Kutch et al. 2014; Rajpurohit  
341 et al. 2016). In *Drosophila melanogaster* females, abdominal pigmentation is  
342 strongly modulated by temperature (Figure 6). This is particularly pronounced  
343 in the more posterior segments (A5, A6 and A7) (Gibert et al. 2000).  
344 Correspondingly, the expression of several genes encoding the enzymes of the

345 cuticular pigment synthesis pathway in the abdominal epidermes of late pupae  
346 and young adults is modulated by temperature (Gibert et al. 2016, 2017).  
347 However, among them, the gene *tan* stands out, being expressed seven times  
348 more in the abdominal epidermis of young females grown at 18°C than 29°C. The  
349 activity of *tan* increases melanin production and genetic experiments showed  
350 that temperature modulation of *tan* expression plays an essential role in thermal  
351 plasticity of female abdominal pigmentation (Gibert et al. 2016). Furthermore,  
352 temperature modulates the activity of the *tan* abdominal enhancer (*t\_MSE*) and  
353 the level of the active histone mark H3K4me3 on *tan* promoter. Interestingly, *tan*  
354 is well known for its involvement in pigmentation variation and divergence in  
355 the *Drosophila melanogaster* species subgroup. In *Drosophila melanogaster*,  
356 independent genome wide association studies performed on European, African  
357 and American populations have identified single nucleotide polymorphisms in  
358 the *t\_MSE* involved in variation of female abdominal pigmentation (Bastide et al.  
359 2013; Dembeck et al. 2015; Endler et al. 2015). In *Drosophila erecta*, female  
360 dimorphic pigmentation is caused by genetic variation in the *t\_MSE* maintained  
361 by balancing selection (Yassin et al. 2016). In *Drosophila santomea*, several  
362 independent loss of function mutations located in the *t\_MSE* have lead to the light  
363 phenotype of this species, whereas in the sister species *Drosophila yakuba* a  
364 functional *t-MSE* enhancer is associated with dark abdominal pigmentation  
365 (Jeong et al. 2008). Thus, in the *Drosophila melanogaster* species subgroup,  
366 plasticity and variation between species of abdominal pigmentation work  
367 through modulation of the same gene and involves genetic changes in the very  
368 same regulatory sequence, *t\_MSE*, that also mediates the effect of temperature on  
369 *tan* expression in *Drosophila melanogaster*.



370

371 **Discussion**

372 These examples demonstrate that several genes showing transcriptional  
373 plasticity in response to environmental changes are differentially expressed  
374 within and/or between species and contribute to phenotypic variation and/or  
375 divergence. Thus, the same genes respond to the environment and are involved  
376 in evolution. It might seem predictable that the same genes are involved in the  
377 plasticity of a trait and the variation/evolution of this trait. However, this is not  
378 so obvious. Indeed, the same phenotypes can sometimes be produced by  
379 different mechanisms. For example, *Drosophila* wing size can be modulated by  
380 both temperature and clinal genetic variation. However, this occurs through  
381 distinct mechanisms: temperature modulates cell size (Azevedo et al. 2002),  
382 whereas clinal genetic variation primarily affects cell number (James et al. 1995).  
383 Similar pigmentation phenotypes can be produced by modulating the expression  
384 of different pigmentation genes (Wittkopp et al. 2003). For example, melanin  
385 production can be increased by up-regulating *tan* or down-regulating *ebony*, that  
386 encode enzymes with antagonist activities (True et al. 2005).

387 In the case of *RCO-A* and *tan*, the variation or divergence in expression is caused  
388 by mutations in the *cis*-regulatory sequences of the genes. This is also likely the  
389 case for *Ubx* during genetic assimilation of the *bithorax* phenotype. Remarkably,  
390 for *tan*, the same *cis*-regulatory sequence mediates, at least partly, the effect of  
391 temperature. It is generally thought that *cis*-regulatory mutations are less  
392 pleiotropic than mutations in *trans*-acting factors (Prud'homme et al. 2007).  
393 Thus, targeting *tan*, a gene encoding a pigmentation enzyme, or *RCO-A*, whose  
394 expression is limited to the sinuses between growing leaf lobes, limit potential

395 deleterious effects. The recurrent involvement of *RCO-A* and *tan* in evolution lead  
396 to their characterization as evolutionary hotspots (Martin and Orgogozo 2013;  
397 Sicard et al. 2014). Why are these two genes targets of recurrent evolution ? For  
398 a given allele, temperature sensitivity of *RCO-A* and *tan* expression leads to  
399 different phenotypes in different environments. Reciprocally, different alleles  
400 will induce different phenotypes in the same environment. It follows that only  
401 some genotypes will produce an optimal phenotype in a given environment. This  
402 may lead to selection of different alleles of these genes in different environments  
403 to reach an optimal phenotype. This is in particular the case if the phenotype  
404 produced by the plastic response is not well adjusted to the environment.  
405 Genetic variation will be selected to reduce or to increase the effect of the  
406 environment. As the environment fluctuates spatially and temporally, adjusting  
407 selection increases genetic variation with functional consequences at these *loci*.  
408 This higher genetic variation facilitates the involvement of those *loci* in  
409 evolution.

410 In the other cases presented in this review, the respective contributions of *cis*  
411 and *trans* variations are not known (*eud-1* in *P. pacificus*, genes modulated by  
412 osmotic challenge in *Fundulus*, *Dll* in *Bicyclus anynana*) but the recent  
413 identification of *trans*-regulators of *eud-1* is a step towards answering this  
414 question (Serobyán et al. 2016). However, what is clear is that evolution has  
415 targeted components of the gene networks regulating the transcriptionally  
416 plastic genes if not these genes themselves. In most cases described in this review,  
417 the gene showing transcriptional plasticity also exhibits genetic variation that  
418 provides substrate for evolution through selection or simply drift. The fact that  
419 the same networks, or even the same genes, are involved in plasticity and

420 evolution contradicts somehow Scheiner's ideas on the decoupling of the genetic  
421 processes controlling the plasticity and the mean of a trait (Scheiner 1993).  
422 In addition, the "flexible stem hypothesis" implies that it is mechanistically  
423 possible for a trait initially plastic to undergo environmental canalization and  
424 become independent of the environment. Several studies have dissected the  
425 mechanisms behind phenotypic plasticity and environmental canalization. Two  
426 major mechanisms have been identified: regulation of chromatin structure and  
427 hormonal signalling/response. It is known that environmental conditions can  
428 modulate DNA methylation (Kucharski et al. 2008), histone post-translational  
429 modifications (Simola et al. 2016; Gibert et al. 2016) or chromatin compaction  
430 (Leung et al. 2014). Thus, stabilization of chromatin structure in an initially  
431 plastic species, could lead to production of a constant phenotype independent of  
432 the environment. For example, artificial down-regulation of DNA methylation in  
433 honey-bees lead to the production of a queen phenotype from larvae that should  
434 have developed as workers because on the diet they fed on (Kucharski et al.  
435 2008). Some alleles might carry variation in their *cis*-regulatory sequences that  
436 promotes the formation of a particular chromatin structure independently of the  
437 environment. This might be the case for *tan* or *Ubx* and selection on this  
438 variation might contribute to modify the sensitivity of their expression to the  
439 environment. Regarding, hormonal regulation, it is known that differences of  
440 plasticity between genotypes could be linked to differences of hormonal  
441 signalling or response (Suzuki and Nijhout 2006). In *Manduca sexta* larvae,  
442 artificial selection on temperature induced pigmentation variation lead to the  
443 production of a polyphenic and a monophenic lines differing by their juvenile  
444 hormone titer and response (Suzuki and Nijhout 2006). Interestingly a similar

445 process applies to differences of plasticity between organs. In *Drosophila*, the  
446 size of late larval brain and adult male genitalia is buffered against nutrient  
447 restriction, whereas the size of other organs decreases under these conditions.  
448 The buffering of genitalia size is caused by repression of *FOXO* expression in the  
449 genital disc which makes it insensitive to the nutritional information provided by  
450 insulin signalling (Tang et al. 2011). The buffering of late larval brain size is  
451 caused by cell-type specific expression of *Alk* and its ligand *Jelly Belly* which  
452 renders neuroblasts insensitive to amino acid and insulin sensing (Cheng et al.  
453 2011). Thus, by modifying the expression of key genes involved in hormonal  
454 signalling or response it is possible to reduce or even abolish the influence of the  
455 environment on a given trait. These studies illustrate what kind of mechanisms  
456 might be involved in the genetic assimilation taking place during the formation  
457 of a monophenic lineage under the “flexible stem hypothesis”.

458 The examples described in this review are compatible with the “flexible stem  
459 hypothesis” (West-Eberhard 2003), which postulate ancestral plasticity followed  
460 by genetic assimilation of alternative morphs in derived lineages. Analysis of  
461 phenotypic plasticity of nematode feeding structures in an evolutionary  
462 framework supports indeed that plasticity was ancestral and that one morph  
463 was stabilized (genetically assimilated) in particular lineages whereas others  
464 have kept plasticity (Kiontke and Fitch 2010). A definite demonstration of the  
465 “flexible stem hypothesis” would be to follow allelic frequencies during genetic  
466 assimilation of a phenotype of interest. This was done in only one of the  
467 examples, but for a deleterious phenotype normally not observed in the wild, *i.e.*  
468 the *bithorax* phenocopies (Gibson and Hogness 1996). Thus, more studies on the  
469 genetic bases of phenotypic plasticity in an evolutionary conceptual framework

470 are needed. In particular, it would be interesting to know whether the example  
471 of *tan* and its  $t_{MSE}$  is just a particular case or whether it is predictive of a more  
472 systematic involvement of the same regulatory sequences in phenotypic  
473 plasticity and species variation and divergence.

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477 **References:**

478

479 Azevedo RBR, French V, Partridge L (2002) Temperature modulates epidermal  
480 cell size in *Drosophila melanogaster*. *J Insect Physiol* 48:231–237.

481 Badyaev AV, Foresman KR (2000) Extreme environmental change and evolution:  
482 stress-induced morphological variation is strongly concordant with  
483 patterns of evolutionary divergence in shrew mandibles. *Proc Biol Sci*  
484 267:371–7.

485 Bastide H, Betancourt A, Nolte V, et al (2013) A Genome-Wide, Fine-Scale Map of  
486 Natural Pigmentation Variation in *Drosophila melanogaster*. *PLoS Genet*  
487 9:e1003534. doi: 10.1371/journal.pgen.1003534

488 Beldade P, Brakefield PM, Long AD (2002) Contribution of *Distal-less* to  
489 quantitative variation in butterfly eyespots. *Nature* 415:315–318. doi:  
490 10.1038/415315a

491 Bento G, Ogawa A, Sommer RJ (2010) Co-option of the hormone-signalling  
492 module dafachronic acid-DAF-12 in nematode evolution. *Nature*  
493 466:494–497. doi: 10.1038/nature09164

494 Bradshaw AD (1965) Evolutionary Significance of Phenotypic Plasticity in Plants.  
495 In: Thoday EWC and JM (ed) *Advances in Genetics*. Academic Press, pp  
496 115–155

497 Brakefield PM, Gates J, Keys D, et al (1996) Development, plasticity and evolution  
498 of butterfly eyespot patterns. *Nature* 384:236–42.

499 Cheng LY, Bailey AP, Leever SJ, et al (2011) Anaplastic lymphoma kinase spares  
500 organ growth during nutrient restriction in *Drosophila*. *Cell* 146:435–447.  
501 doi: 10.1016/j.cell.2011.06.040

- 502 Dembeck LM, Huang W, Magwire MM, et al (2015) Genetic Architecture of  
503 Abdominal Pigmentation in *Drosophila melanogaster*. PLoS Genet  
504 11:e1005163. doi: 10.1371/journal.pgen.1005163
- 505 Dworkin I (2005) Towards a genetic architecture of cryptic genetic variation and  
506 genetic assimilation: the contribution of K. G. Bateman. J Genet 84:223–  
507 226.
- 508 Endler L, Betancourt AJ, Nolte V, Schlötterer C (2015) Reconciling Differences in  
509 Pool-GWAS between Populations: A Case Study of Female Abdominal  
510 Pigmentation in *Drosophila melanogaster*. Genetics. doi:  
511 10.1534/genetics.115.183376
- 512 Fedorka KM, Copeland EK, Winterhalter WE (2013) Seasonality influences  
513 cuticle melanization and immune defense in a cricket: support for a  
514 temperature-dependent immune investment hypothesis in insects. J Exp  
515 Biol 216:4005–10. doi: 10.1242/jeb.091538
- 516 Ghalambor CK, Hoke KL, Ruell EW, et al (2015) Non-adaptive plasticity  
517 potentiates rapid adaptive evolution of gene expression in nature. Nature  
518 525:372–375. doi: 10.1038/nature15256
- 519 Gibert J-M, Mouchel-Vielh E, De Castro S, Peronnet F (2016) Phenotypic Plasticity  
520 through Transcriptional Regulation of the Evolutionary Hotspot Gene *tan*  
521 in *Drosophila melanogaster*. PLoS Genet 12:e1006218. doi:  
522 10.1371/journal.pgen.1006218
- 523 Gibert J-M, Mouchel-Vielh E, Peronnet F (2017) Modulation of yellow expression  
524 contributes to thermal plasticity of female abdominal pigmentation in  
525 *Drosophila melanogaster*. Sci Rep 7:43370. doi: 10.1038/srep43370
- 526 Gibert P, Moreteau B, David JR (2000) Developmental constraints on an adaptive  
527 plasticity: reaction norms of pigmentation in adult segments of  
528 *Drosophila melanogaster*. Evol Dev 2:249–60.
- 529 Gibson G, Hogness DS (1996) Effect of polymorphism in the *Drosophila*  
530 regulatory gene *Ultrabithorax* on homeotic stability. Science 271:200–3.
- 531 Gilbert SF (2001) Ecological developmental biology: developmental biology  
532 meets the real world. Dev Biol 233:1–12. doi: 10.1006/dbio.2001.0210
- 533 James AC, Azevedo RB, Partridge L (1995) Cellular basis and developmental  
534 timing in a size cline of *Drosophila melanogaster*. Genetics 140:659–66.
- 535 Jeong S, Rebeiz M, Andolfatto P, et al (2008) The evolution of gene regulation  
536 underlies a morphological difference between two *Drosophila* sister  
537 species. Cell 132:783–93.
- 538 Johannsen W (1911) The genotype conception of heredity. Am Nat XLV:129–159.

- 539 Kiontke K, Fitch DHA (2010) Phenotypic plasticity: different teeth for different  
540 feasts. *Curr Biol* CB 20:R710-712. doi: 10.1016/j.cub.2010.07.009
- 541 Kucharski R, Maleszka J, Foret S, Maleszka R (2008) Nutritional control of  
542 reproductive status in honeybees via DNA methylation. *Science*  
543 319:1827-30.
- 544 Kutch IC, Sevgili H, Wittman T, Fedorka KM (2014) Thermoregulatory strategy  
545 may shape immune investment in *Drosophila melanogaster*. *J Exp Biol*  
546 217:3664-9. doi: 10.1242/jeb.106294
- 547 Laland K, Uller T, Feldman M, et al (2014) Does evolutionary theory need a  
548 rethink? *Nature* 514:161-164. doi: 10.1038/514161a
- 549 Laland KN, Uller T, Feldman MW, et al (2015) The extended evolutionary  
550 synthesis: its structure, assumptions and predictions. *Proc Biol Sci*  
551 282:20151019. doi: 10.1098/rspb.2015.1019
- 552 Leung A, Parks BW, Du J, et al (2014) Open chromatin profiling in mice livers  
553 reveals unique chromatin variations induced by high fat diet. *J Biol Chem.*  
554 doi: 10.1074/jbc.M114.581439
- 555 Martin A, Orgogozo V (2013) The Loci of repeated evolution: a catalog of genetic  
556 hotspots of phenotypic variation. *Evolution* 67:1235-50. doi:  
557 10.1111/evo.12081
- 558 Michie LJ, Mallard F, Majerus ME, Jiggins FM (2010) Melanic through nature or  
559 nurture: genetic polymorphism and phenotypic plasticity in *Harmonia*  
560 *axyridis*. *J Evol Biol* 23:1699-707. doi: 10.1111/j.1420-  
561 9101.2010.02043.x
- 562 Moczek AP, Sultan S, Foster S, et al (2011) The role of developmental plasticity in  
563 evolutionary innovation. *Proc Biol Sci*. doi: 10.1098/rspb.2011.0971
- 564 Monteiro A, Chen B, Ramos DM, et al (2013) Distal-less regulates eyespot  
565 patterns and melanization in *Bicyclus* butterflies. *J Exp Zool B Mol Dev*  
566 *Evol* 320:321-331. doi: 10.1002/jez.b.22503
- 567 Muschick M, Barluenga M, Salzburger W, Meyer A (2011) Adaptive phenotypic  
568 plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in  
569 adaptive radiation. *BMC Evol Biol* 11:116. doi: 10.1186/1471-2148-11-  
570 116
- 571 Pfennig DW, Wund MA, Snell-Rood EC, et al (2010) Phenotypic plasticity's  
572 impacts on diversification and speciation.
- 573 Pigliucci M (2001) *Phenotypic Plasticity, Beyond Nature and Nurture*. Baltimore  
574 and London
- 575 Prud'homme B, Gompel N, Carroll SB (2007) Emerging principles of regulatory  
576 evolution. *Proc Natl Acad Sci U A* 104 Suppl 1:8605-12.

- 577 Ragsdale EJ, Müller MR, Rödelsperger C, Sommer RJ (2013) A developmental  
578 switch coupled to the evolution of plasticity acts through a sulfatase. *Cell*  
579 155:922–933. doi: 10.1016/j.cell.2013.09.054
- 580 Rajpurohit S, Richardson R, Dean J, et al (2016) Pigmentation and fitness trade-  
581 offs through the lens of artificial selection. *Biol Lett*. doi:  
582 10.1098/rsbl.2016.0625
- 583 Scheiner SM (1993) Plasticity as a Selectable Trait: Reply to Via. *Am Nat*  
584 142:371–373.
- 585 Schlichting CD, Pigliucci M (1993) Control of phenotypic plasticity via regulatory  
586 genes. *Am Nat* 142:366–370. doi: 10.1086/285543
- 587 Schmalhausen II (1949) *Factors of Evolution, The Theory of Stabilizing Selection.*  
588 The University of Chicago Press, Chicago and London
- 589 Schneider RF, Meyer A (2017) How plasticity, genetic assimilation and cryptic  
590 genetic variation may contribute to adaptive radiations. *Mol Ecol* 26:330–  
591 350. doi: 10.1111/mec.13880
- 592 Schwander T, Leimar O (2011) Genes as leaders and followers in evolution.  
593 *Trends Ecol Evol* 26:143–151. doi: 10.1016/j.tree.2010.12.010
- 594 Serobyian V, Xiao H, Namdeo S, et al (2016) Chromatin remodelling and  
595 antisense-mediated up-regulation of the developmental switch gene *eud-*  
596 *1* control predatory feeding plasticity. *Nat Commun* 7:12337. doi:  
597 10.1038/ncomms12337
- 598 Shirai LT, Saenko SV, Keller RA, et al (2012) Evolutionary history of the  
599 recruitment of conserved developmental genes in association to the  
600 formation and diversification of a novel trait. *BMC Evol Biol* 12:21. doi:  
601 10.1186/1471-2148-12-21
- 602 Sicard A, Thamm A, Marona C, et al (2014) Repeated evolutionary changes of leaf  
603 morphology caused by mutations to a homeobox gene. *Curr Biol* 24:1880–  
604 6. doi: 10.1016/j.cub.2014.06.061
- 605 Simola DF, Graham RJ, Brady CM, et al (2016) Epigenetic (re)programming of  
606 caste-specific behavior in the ant *Camponotus floridanus*. *Science*. doi:  
607 10.1126/science.aac6633
- 608 Susoy V, Ragsdale EJ, Kanzaki N, Sommer RJ (2015) Rapid diversification  
609 associated with a macroevolutionary pulse of developmental plasticity.  
610 *eLife*. doi: 10.7554/eLife.05463
- 611 Suzuki Y, Nijhout HF (2006) Evolution of a polyphenism by genetic  
612 accommodation. *Science* 311:650–2.



- 613 Tang HY, Smith-Caldas MS, Driscoll MV, et al (2011) FOXO regulates organ-  
614 specific phenotypic plasticity in *Drosophila*. *PLoS Genet* 7:e1002373. doi:  
615 10.1371/journal.pgen.1002373
- 616 True JR, Yeh SD, Hovemann BT, et al (2005) *Drosophila tan* Encodes a Novel  
617 Hydrolase Required in Pigmentation and Vision. *PLoS Genet* 1:e63.
- 618 Via S (1993) Adaptive phenotypic plasticity: target or by-product of selection in a  
619 variable environment? *Am Nat* 142:352–365. doi: 10.1086/285542
- 620 Via S, Gomulkiewicz R, de Jong G, et al (1995) Adaptive phenotypic plasticity:  
621 consensus and controversy. *TREE* 10:212–217.
- 622 Waddington CH (1952) Selection of the genetic basis for an acquired character.  
623 *Nature* 169:278.
- 624 Waddington CH (1956) Genetic Assimilation of the Bithorax Phenotype.  
625 *Evolution* 10:1–13. doi: 10.2307/2406091
- 626 Waddington CH (1959) Canalization of development and genetic assimilation of  
627 acquired characters. *Nature* 183:1654–5.
- 628 West-Eberhard MJ (2003) *Developmental plasticity and evolution*. Oxford  
629 University Press, New York
- 630 West-Eberhard MJ (2005) Developmental plasticity and the origin of species  
631 differences. *Proc Natl Acad Sci U A* 102 Suppl 1:6543–9.
- 632 Whitehead A, Roach JL, Zhang S, Galvez F (2011) Genomic mechanisms of  
633 evolved physiological plasticity in killifish distributed along an  
634 environmental salinity gradient. *Proc Natl Acad Sci U A* 108:6193–8. doi:  
635 10.1073/pnas.1017542108
- 636 Whitehead A, Roach JL, Zhang S, Galvez F (2012) Salinity- and population-  
637 dependent genome regulatory response during osmotic acclimation in the  
638 killifish (*Fundulus heteroclitus*) gill. *J Exp Biol* 215:1293–1305. doi:  
639 10.1242/jeb.062075
- 640 Wittkopp PJ, Beldade P (2009) Development and evolution of insect  
641 pigmentation: genetic mechanisms and the potential consequences of  
642 pleiotropy. *Semin Cell Dev Biol* 20:65–71.
- 643 Wittkopp PJ, Stewart EE, Arnold LL, et al (2009) Intraspecific polymorphism to  
644 interspecific divergence: genetics of pigmentation in *Drosophila*. *Science*  
645 326:540–4. doi: 10.1126/science.1176980
- 646 Wittkopp PJ, Williams BL, Selegue JE, Carroll SB (2003) *Drosophila* pigmentation  
647 evolution: divergent genotypes underlying convergent phenotypes. *Proc*  
648 *Natl Acad Sci U A* 100:1808–13.

- 649 Woltereck R (1909) Weitere experimentelle unters"uchungen "uber  
650 artver"anderung, speziell "uber das wesen quantitativer artunterschiede  
651 bei daphniden. Verhandlungen Dtsch. Zooligischen Ges. 110–172.
- 652 Wund MA, Baker JA, Clancy B, et al (2008) A test of the "flexible stem" model of  
653 evolution: ancestral plasticity, genetic accommodation, and  
654 morphological divergence in the threespine stickleback radiation. *Am Nat*  
655 172:449–462. doi: 10.1086/590966
- 656 Yassin A, Bastide H, Chung H, et al (2016) Ancient balancing selection at tan  
657 underlies female colour dimorphism in *Drosophila erecta*. *Nat Commun*  
658 7:10400. doi: 10.1038/ncomms10400
- 659

660 **Figure legends:**

661

662 **Figure 1:** Above: wild-type *Drosophila melanogaster* female. Bellow: female  
663 *Drosophila* showing a *bithorax* phenotype obtained after genetic assimilation of  
664 the *bithorax* phenocopy initially induced by ether treatment of the embryo  
665 (modified after (Waddington 1956)). The post-notum is replaced by an  
666 additional meso-notum and halteres are replaced by rudimentary wings.

667

668 **Figure 2:** Left: the network of rivers flowing into the Chesapeake-bay, in north-  
669 east America, constitutes a range of water habitat with different salinity going  
670 from pure sea water (dark blue) to pure fresh water (red), modified after  
671 (Whitehead et al. 2011). Top right, populations of Atlantic killifish (*Fundulus*  
672 *heteroclitus*) are locally adapted to particular salinities but are also able to  
673 quickly adjust to osmotic challenges. Bottom right: modification of gill  
674 epithelium after transfer form sea water to fresh water (after (Whitehead et al.  
675 2012)). In sea water (32 ppt), the apical surface of the gill epithelium is made of  
676 pavement cells (red asterisk) interspersed with mitochondrion-rich cells  
677 possessing apical crypt with small surface area (green arrowhead). Exposure to  
678 fresh water (0.1 ppt) leads to an enlargement of the apical surface of  
679 mitochondrion-rich cells (green arrowhead).

680

681 **Figure 3:** Top: natural populations of the satyrine butterfly *Bicyclus anynana*  
682 show two seasonal morphs, the dry season morph (left) and the wet season  
683 morph (right) that can be produced in the lab by rearing the larvae at 17°C or  
684 27°C, respectively. Middle and bottom: the dry season and the wet season

685 morphs can be fixed by artificial selection leading to a LOW line (middle) or a  
686 HIGH line (bottom) with very limited plasticity (after (Brakefield et al. 1996)).

687

688 **Figure 4:** The diplogastrid nematode *Pristionchus pacificus* displays two  
689 alternative tooth morphs: a wide one, eury stomatous (Eu, left) or a narrow one  
690 stenostomatous (St, right), that are produced after an irreversible decision  
691 during larval development (after (Kiontke and Fitch 2010)). The ratio of these  
692 two morphs is variable between strains and is modulated by environmental  
693 factors such as crowding and starvation.

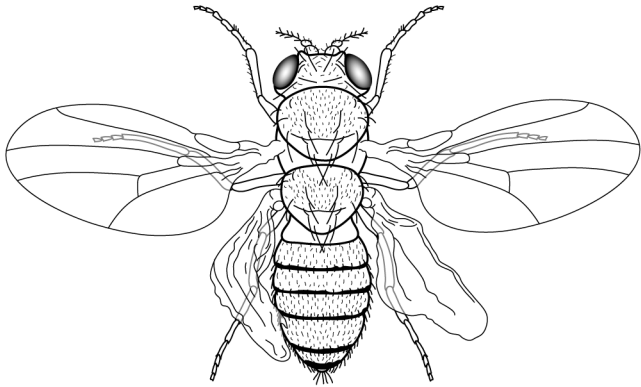
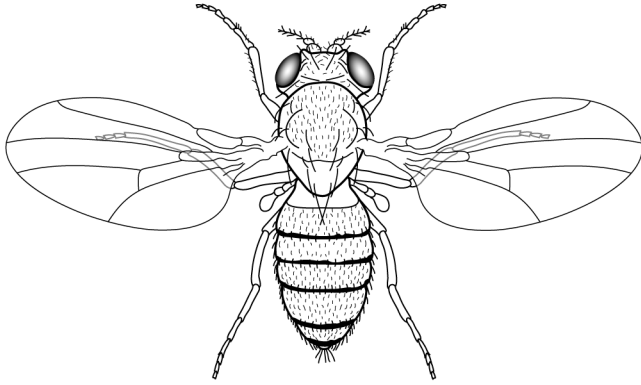
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695 **Figure 5:** *Capsella grandiflora* and *Capsella rubella* differ regarding the  
696 dissection of their leaf margins. This difference was mapped to the *RCO-A locus*.  
697 Above nearly isogenic lines (NIL) differing only in the *RCO-A locus* region. The  
698 line with the *C. grandiflora* allele (*NILgg*) (left) does not show any leaf margin  
699 dissection at 22°C contrary to the line with the *C. rubella* allele (*NILrr*) (right).  
700 Lower temperature (16°C) induces (*NILgg*) or increases (*NILrr*) leaf margin  
701 dissection.

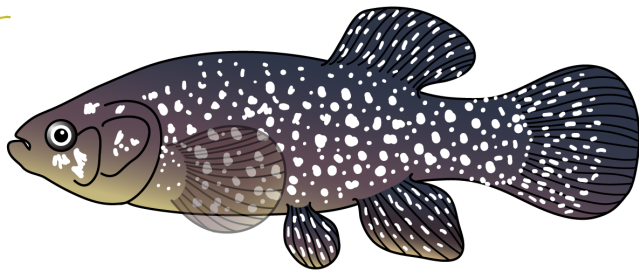
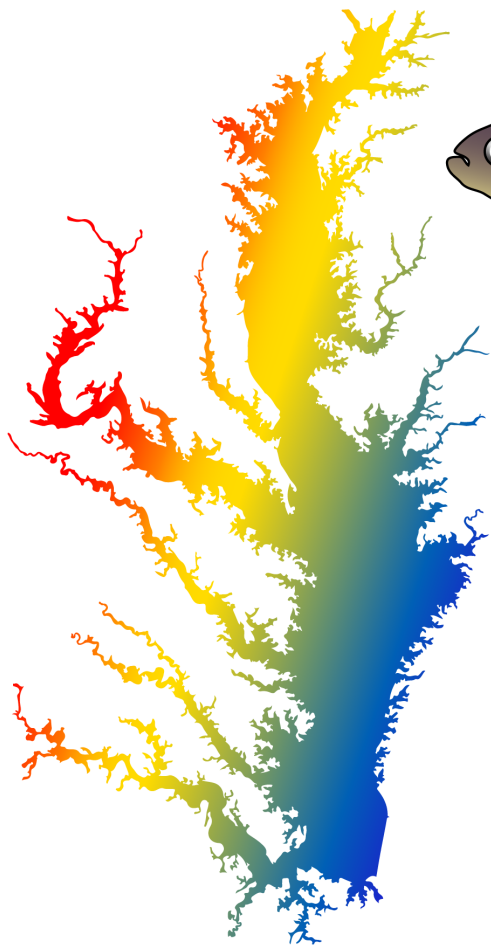
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703 **Figure 6:** Abdominal cuticles of females *Drosophila melanogaster* from an inbred  
704 line (*w<sup>1118</sup>*) grown at 18°C (left) or 29°C (right). Female abdominal pigmentation  
705 is sensitive to temperature during development in particular in the most  
706 posterior segments (A5, A6 and A7). Anterior is up, ventral is left (after (Gibert  
707 et al. 2016)).

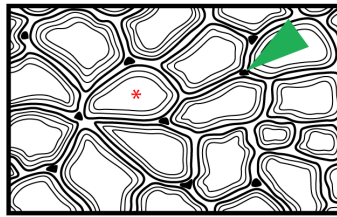
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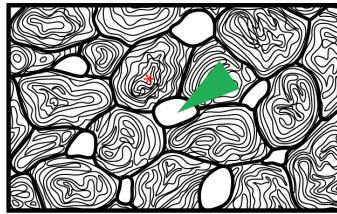
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32 ppt, Pre-transfer

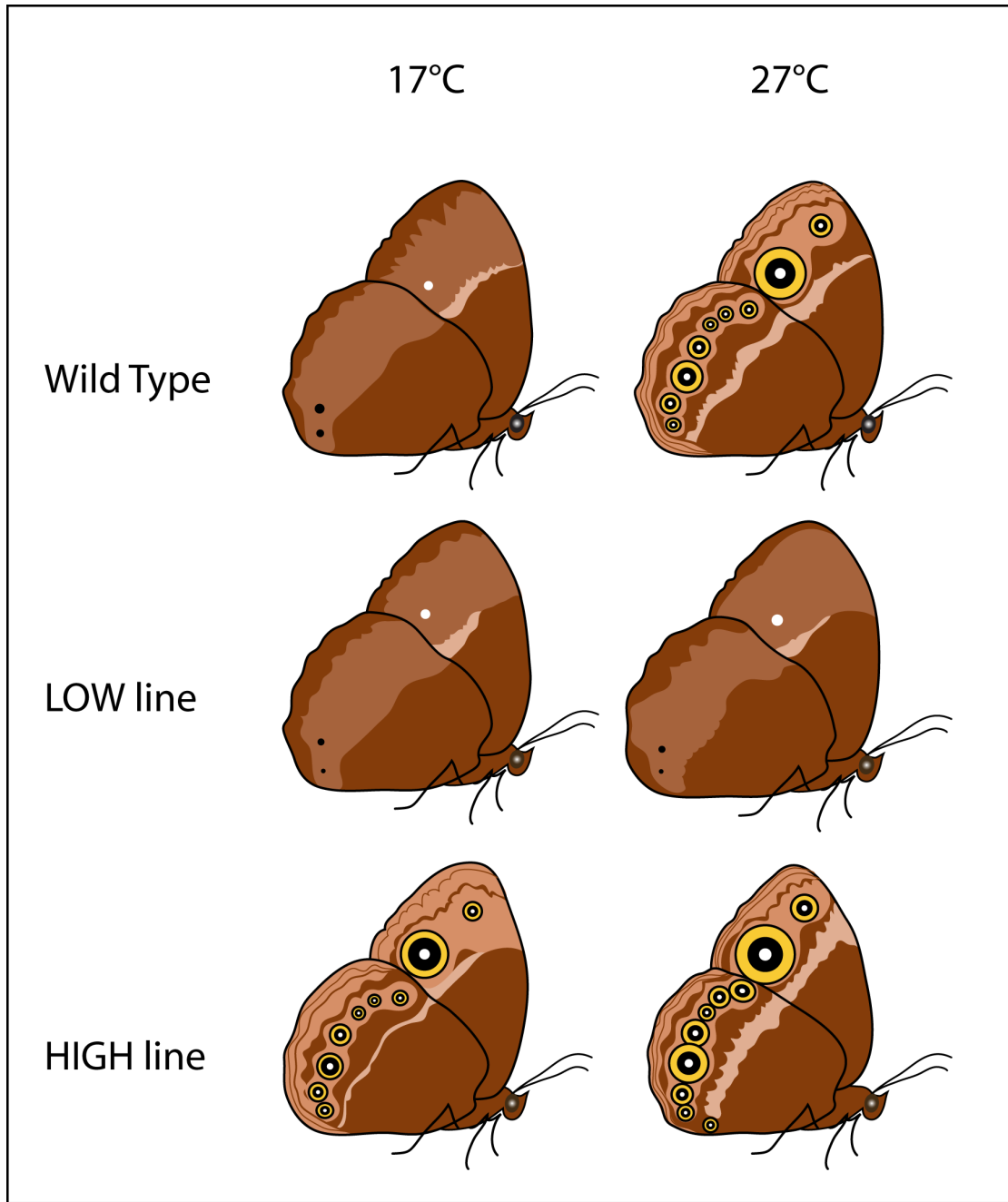


0.1 ppt, 1 day post transfer



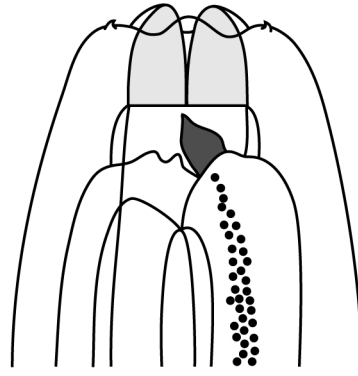
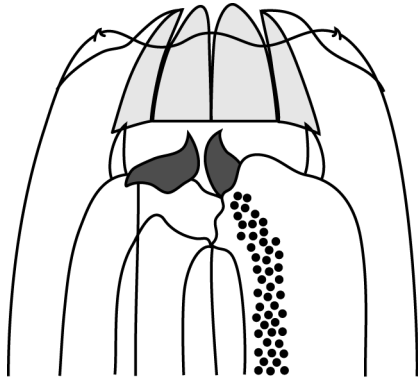
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714

Eurystomatous (Eu)

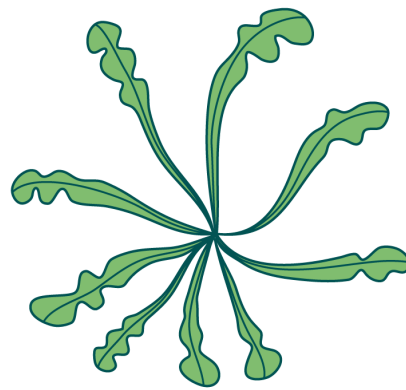
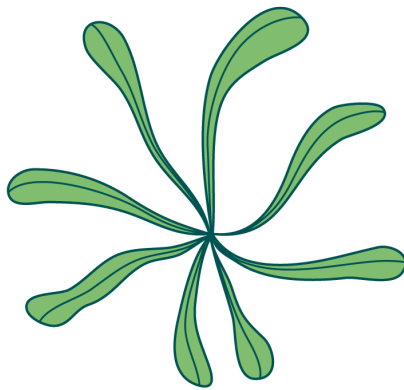
Stenostomatous (St)

715

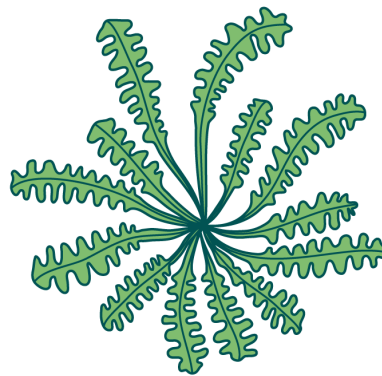
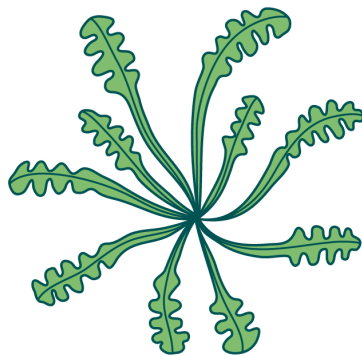
*NILgg*

*NILrr*

22°C



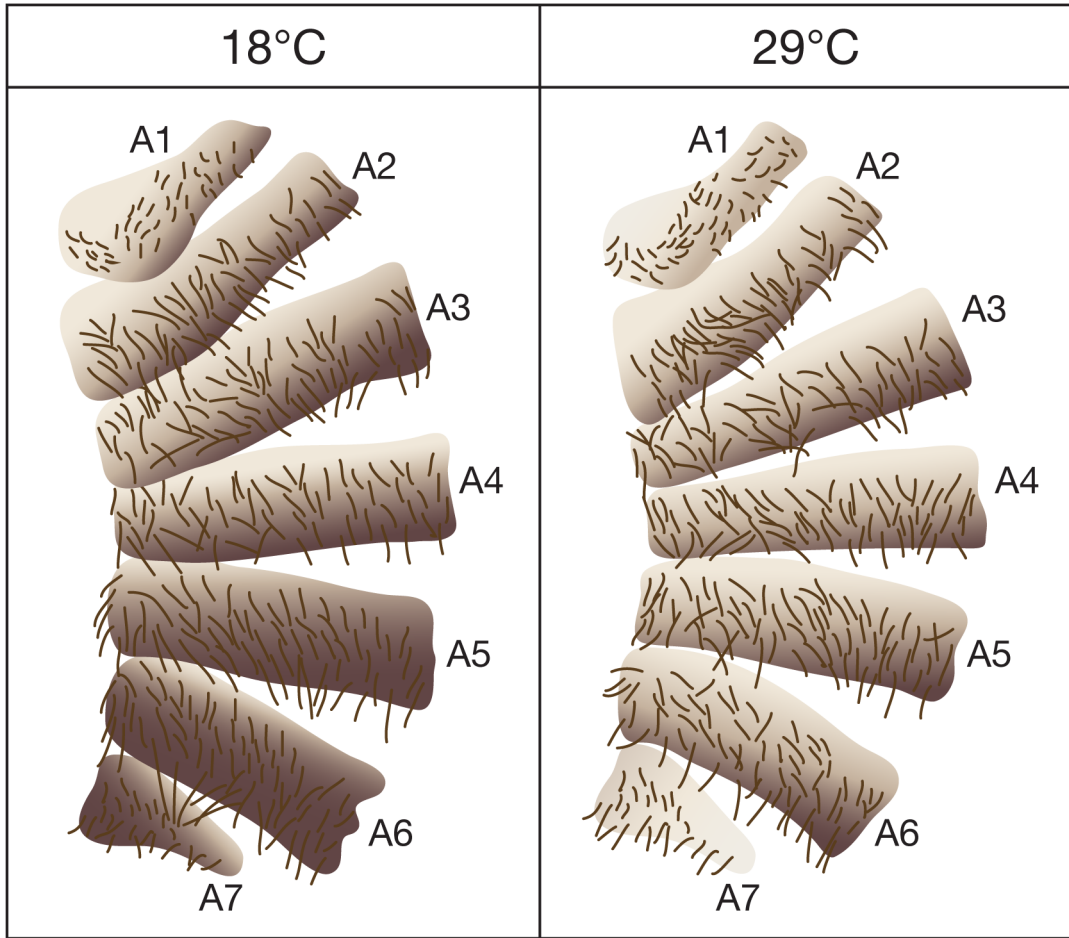
16°C



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720