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### The sources of adaptive variation

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1 **The sources of adaptive variation**

2

3 Deborah Charlesworth<sup>1</sup>, Nicholas H. Barton<sup>2</sup>, Brian Charlesworth<sup>1</sup>

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5 <sup>1</sup>Institute of Evolutionary Biology, School of Biological Sciences, University of  
6 Edinburgh, Charlotte Auerbach Rd., Edinburgh EH9 3FL, UK

7

8 <sup>2</sup>Institute of Science and Technology Austria, Klosterneuburg 3400, Austria

9

10 **Author for correspondence:**

11 Brian Charlesworth

12 Email: Brian.Charlesworth@ed.ac.uk

13

14 Phone: 0131 650 5751

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24

25

26

27

28 **Abstract**

29 The role of natural selection in the evolution of adaptive phenotypes has undergone  
30 constant probing by evolutionary biologists, employing both theoretical and empirical  
31 approaches. As Darwin noted, natural selection can act together with other processes,  
32 including random changes in the frequencies of phenotypic differences that are not  
33 under strong selection, and changes in the environment, which may reflect  
34 evolutionary changes in the organisms themselves. As understanding of genetics  
35 developed after 1900, the new genetic discoveries were incorporated into evolutionary  
36 biology. The resulting general principles were summarised by Julian Huxley in his  
37 1942 book *Evolution, The Modern Synthesis*. Here, we examine how recent advances  
38 in genetics, developmental biology and molecular biology, including epigenetics,  
39 relate to today's understanding of the evolution of adaptations. We illustrate how  
40 careful genetic studies have repeatedly shown that apparently puzzling results in a  
41 wide diversity of organisms involve processes that are consistent with neo-  
42 Darwinism. They do not support important roles in adaptation for processes such as  
43 directed mutation or the inheritance of acquired characters, and therefore no radical  
44 revision of our understanding of the mechanism of adaptive evolution is needed.  
45

46 *“Darwinism has been under constant scrutiny ever since On the Origin of Species*  
47 *was published. The theory of evolution by natural selection, based on variation and*  
48 *selection, provided a hitherto unparalleled explanation of life's diversity and change,*  
49 *invoking no forces other than simple biological ones, such as heredity and mutation.*  
50 *One of the main ideas that derive from Darwinism – and, in my view, one of the most*  
51 *powerful ideas in the history of science – is that adaptation and design can arise*  
52 *without any ... guiding hand” [1].*

53

## 54 **1. Introduction**

55 During the 1930s and 1940s, the findings of classical and quantitative genetics were  
56 integrated into general evolutionary biology, in response to the population genetic  
57 models of evolutionary processes pioneered by Fisher, Haldane and Wright. The  
58 Modern Synthesis of evolution (MS) was named by Julian Huxley [2] to emphasise  
59 the wide acceptance of its principles as a framework for understanding the  
60 mechanisms of evolution, and for interpreting data on a wide range of biological  
61 phenomena. Its basic ideas remain central to contemporary biology, despite enormous  
62 advances over the past 80 years, especially those connected with the rise of molecular  
63 biology.

64 The core tenet of the MS is that adaptive evolution is due to natural selection  
65 acting on heritable variability that originates through accidental changes in the genetic  
66 material. Such mutations are random in the sense that they arise without reference to  
67 their advantages or disadvantages (i.e. their fitness effects), although their phenotypic  
68 effects are necessarily constrained by organisms' developmental systems [3, 4], as  
69 was recognised by the founders of the MS, e.g. [5]. Because this viewpoint asserts  
70 that natural selection acts to increase the frequencies of advantageous variants within  
71 populations, it is often referred to as neo-Darwinism.

72 Processes other than natural selection and mutation were, however, also  
73 included in the MS – most notably genetic drift (random fluctuations in the  
74 frequencies of variants in finite populations), which is the basis of the neutral theory  
75 of molecular evolution [6] that is widely used as a null model for interpreting data on  
76 DNA sequence variation and evolution. But a random process such as drift cannot  
77 explain adaptation, except when it acts in conjunction with selection, as in Wright's  
78 shifting balance theory [7]. A powerful theoretical argument for the predominant role  
79 of selection in adaptive evolution was provided by Fisher's discovery that (in modern

80 terminology) the evolutionary fate of a new mutation is controlled by the product of  
81 the effective population size ( $N_e$ ) and the intensity of selection that it experiences [8].  
82 A selection intensity of the order of the reciprocal of  $N_e$  can prevent a harmful  
83 mutation from spreading, or allow selection to promote the spread of a beneficial  
84 mutation. Even when selection is weak, it is therefore likely to dominate over drift  
85 and mutation pressure for most traits, except in species with very small population  
86 sizes.

87         There has, however, been a long history of proposed alternatives to the MS,  
88 including Goldschmidt's saltational theory of evolution by 'macromutations' creating  
89 coordinated adaptive phenotypes with multiple differences from their progenitors [9],  
90 and the Lysenkoist advocacy of the inheritance of acquired characters that dominated  
91 biology in the Soviet Union and its satellites for many years [10, 11]. In the 1970s and  
92 1980s, advocates of punctuated equilibria, developmental constraints and molecular  
93 drive again challenged the MS [3], and claims for the Lamarckian inheritance of  
94 acquired characters were renewed [12]. These challenges were quickly shown not to  
95 raise serious difficulties, and the appearance of inheritance of acquired characters in  
96 immune responses was explained in terms of other processes [12]. Recently, however,  
97 several challenges to the MS have again been made, resurrecting some of these old  
98 criticisms and adding new ones. It is claimed that neo-Darwinism has overlooked  
99 important evolutionary factors, and must be supplemented by a self-proclaimed  
100 'Extended Evolutionary Synthesis' (EES) [13-15], which "*is not just an extension of*  
101 *the MS, but a distinctively different framework for understanding evolution*" [14].  
102 Some even propose that the MS needs to be replaced, e.g. [16].

103         In the present review, we evaluate one aspect of such claims: the central  
104 question of the source of the variability involved in adaptive evolution. Other aspects  
105 have been studied within the framework of the MS, and therefore do not seriously  
106 challenge neo-Darwinism. These include the roles of developmental constraints and  
107 phenotypic plasticity in evolution, and interactions of organisms with their  
108 environment in ways that influence their subsequent evolution, 'niche construction'  
109 [3, 4, 17, 18]. We therefore focus on empirical evidence relevant to the claim that  
110 natural selection acting on 'random' mutations is inadequate to explain adaptive  
111 evolution [14-16, 19-21] (see also the website [www.thethirdwayofevolution.com](http://www.thethirdwayofevolution.com)). To  
112 avoid circularity, we define an adaptation as a trait that appears to be designed to fulfil  
113 an organismal purpose.

114 We critically examine the current status of evidence for proposed alternative  
115 mechanisms for generating adaptively useful variation, especially the inheritance of  
116 acquired adaptive characters and directed mutation. Our motivation for focussing on  
117 this topic is that neo-Darwinian evolution requires the transformation of a population  
118 over time as a result of natural selection. If variants tended systematically to arise  
119 when they are adaptive, many or all individuals in a population could acquire  
120 adaptations without the need for selection; this would indeed constitute a serious  
121 challenge to the MS. As John Maynard Smith once said “.. *the question of the origin*  
122 *of hereditary variation remains central to evolutionary biology, if only because*  
123 *Lamarck’s theory is the only alternative to Darwinism that has been suggested*” [22,  
124 p.91].

125 Overall, based on recent research papers and reviews that exhaustively  
126 examine the proposed alternative processes generating variation, we find no evidence  
127 to support such a challenge. Indeed, modern research in population genomics is  
128 providing ever-stronger evidence for the footprints of natural selection [23-25].

129

## 130 **2. Unconventional inheritance systems and adaptive evolution**

131

132 “*Before we rewrite the textbooks, divert funding initiatives, refocus our disease*  
133 *intervention strategies, or alter our view of neo-Darwinian biology, it is our*  
134 *obligation to attempt these simple tests to assure ourselves that we are not chasing a*  
135 *ghost*” [26].

136 The EES and other recent critiques of neo-Darwinism claim that new discoveries  
137 undermine its core premise that random mutations are the source of the variation on  
138 which natural selection acts. Specifically, it is proposed that ‘unconventional’ modes  
139 of inheritance such as ‘epigenetic’ inheritance permit the transmission of acquired,  
140 adaptive characters [19, 21]. Point (vi) of Table 3 in [15] states that “*in addition to*  
141 *selection, adaptive variants are propagated through repeated environmental*  
142 *induction, non-genetic inheritance, learning and cultural transmission*”; point (vii)  
143 proposes that the induction of functional variants may help explain rapid phenotypic  
144 evolution.

145 We will not discuss cultural transmission, since this way of passing  
146 information between generations does not involve heritable processes as normally  
147 understood in biology, although of course cultural practices may affect biological

148 evolution in the small minority of species with advanced social behaviour [4]. Instead,  
149 we focus on mechanisms that might allow adaptive phenotypic traits to become  
150 expressed by all or most members of populations, without a neo-Darwinian  
151 evolutionary process.

152

### 153 **(a) Classical genetics and inheritance**

154 The MS was based on the rules of inheritance discovered by classical genetics, which  
155 apply to any stably inherited type of variant associated with a chromosome, whether  
156 or not it involves a DNA sequence change. Early 20<sup>th</sup> century genetics showed that  
157 most genetic variants associated with major phenotypic differences in animals, plants  
158 and fungi are stably and biparentally inherited (Mendelian inheritance), and  
159 chromosomally located, as was eloquently summarised by H.J. Muller [27]. It was  
160 subsequently shown that inheritance in bacteria and viruses obeys fundamentally  
161 similar rules [28]. Matrilineal inheritance also occurs, involving the transmission of  
162 variants in plastid and mitochondrial genomes [29], or of cytoplasmic endosymbionts  
163 such as *Wolbachia* [30]. The multifactorial theory of quantitative trait variability, and  
164 its experimental validation, showed that Mendelian variants with small phenotypic  
165 effects underlie heritable quantitative trait variation, acting together with non-genetic  
166 factors [31]. These discoveries allowed population geneticists to model evolutionary  
167 changes within populations; their results convinced biologists that natural selection  
168 was highly effective as an evolutionary mechanism, contrary to other views that had  
169 prevailed into the 1930s [31].

170         Some rare cases of unstable inheritance of mutant phenotypes, however,  
171 initially remained puzzling. It is now known that these are often caused by disruptions  
172 of gene function by insertions of transposable elements (TEs), whose excision can  
173 sometimes restore the wild-type allele [32]. Because most TE insertions excise very  
174 rarely, such mutations mostly follow Mendel's laws – indeed, many of the classical  
175 mutations in *Drosophila* genetics [33], and in the sweet peas studied by Mendel,  
176 involved TE insertions [34].

177         In recent years, the term 'genetic inheritance' has come to mean the  
178 transmission of alterations in the DNA sequence (or RNA sequence, in the case of  
179 some viral genomes), as distinct from a heterogeneous set of phenomena that do not  
180 involve such alterations. In the next sections, we outline current knowledge about  
181 these other processes, which have come to be called 'epigenetic' inheritance, and

182 consider their implications for the validity of the MS (see [35] and [36] for earlier  
183 discussions of this issue).

184

### 185 **(b) Epigenetic inheritance processes**

186 We define epigenetic inheritance as the transmission of epigenetic information  
187 between generations, distinguishing between two types of processes. The first (type 1)  
188 includes variants (epialleles) involving chromatin marks such as methylation of DNA  
189 basepairs and histones. Epialleles are defined as ‘marked’ allelic forms whose  
190 phenotypic effects (if any) depend on their epigenetic states, rather than on DNA  
191 sequence differences. Type 2 involves changes associated with regulatory molecules  
192 such as small interfering RNAs, which can be transmitted through the gametes,  
193 resulting in non-Mendelian inheritance. Both types can be associated with phenotypic  
194 effects, and could potentially allow characteristics acquired during the life of an  
195 individual to be inherited by its descendants, in the absence of any DNA sequence  
196 variants [19, 21].

197 In examining the role of type 1 epigenetic inheritance in evolution, we  
198 distinguish meiotically heritable but potentially reversible chromatin alterations at a  
199 site, without associated DNA sequence differences, from alterations controlled by  
200 sequence variants, either at the site or elsewhere in the genome. It can be difficult to  
201 determine whether epigenetic marks are transmitted across generations independently  
202 of DNA sequence differences [37, 38].

203 Several situations that are sometimes regarded as epigenetic inheritance do not  
204 involve transmission of informational macromolecules across generations, so that part  
205 of the controversy about the importance of epigenetic inheritance is semantic [26].  
206 Here, we exclude phenomena such as direct effects of parental condition on the  
207 offspring in organisms like mammals, and maternal effects mediated through  
208 provisioning of the egg cytoplasm. Chemical treatments can pass from maternal  
209 parents and affect the progeny while they are developing, including the germ lines of  
210 both male and female progeny, so that effects can occur two or even three generations  
211 after exposure [39]. Both genetically and environmentally caused maternal effects  
212 have long been included in models of evolutionary processes [40, 41], and do not  
213 challenge neo-Darwinism.



214           There are, however, several questions concerning the evolutionary  
215 significance of epigenetic inheritance, some of which remain to be answered by future  
216 research.

- 217       • For how many generations do inherited epigenetic marks persist, and are they  
218       stable enough to affect evolutionary processes? For example, if advantageous  
219       to individuals, can they spread through a population and become almost fixed,  
220       or do they change back to the unmarked state too frequently for these marks to  
221       maintain adaptation? In evolutionary terms, what are the forward and  
222       backward mutation rates?
- 223       • What kinds of sequences in genomes are affected by these phenomena, and  
224       what fraction of the genome do they represent? Specifically, are the ‘core  
225       genes’ of organisms affected, or are epigenetic modifications largely confined  
226       to transposable element sequences or to other types of repetitive sequences?  
227       Are these effects due to processes that evolved to defend genomes against  
228       selfish ‘genomic parasites’ (particularly in the germ line)?
- 229       • Do epiallelic variants affect phenotypes?
- 230       • Does epigenetic inheritance contribute to variability in quantitative characters  
231       of evolutionary importance?
- 232       • Are epigenetically inherited changes an important source of adaptive change,  
233       compared to DNA sequence change?

234

235           In the following sections of the paper, we discuss several phenomena that are  
236 relevant to these questions.

237

### 238 **3. Experimental evidence for epigenetic inheritance**

239

#### 240 **(a) Epigenetic systems in defence against transposable elements and** 241 **viruses**

242 An initially very puzzling exception to Mendelian inheritance was provided by the  
243 phenomenon of hybrid dysgenesis, discovered in *Drosophila melanogaster* in the late  
244 1970s, and which is now known to involve high rates of movement of certain types of  
245 transposable elements (TEs) [42, 43]. TEs can cause harmful effects on their hosts  
246 when they insert into coding or regulatory sequences. Other effects include

247 chromosome breakage when TEs insert or excise, and the production of chromosome  
248 rearrangements by recombination between homologous TEs in different genome  
249 locations. These harmful fitness effects of TEs often keep their frequencies at  
250 potential insertion sites low in natural populations, and generate selection on their  
251 hosts to suppress their movement [43, 44].

252         Hybrid dysgenesis occurs when a male that carries members of certain TE  
253 families is crossed with a female that lacks them [42, 43]. In the eggs of such mothers,  
254 the defence system in the cytoplasm fails to inactivate the TEs introduced from the  
255 father, which therefore transpose very actively in the offspring, causing sterility.  
256 Susceptibility to hybrid dysgenesis can be transmitted through the maternal lineage  
257 over several generations. The system whose failure causes hybrid dysgenesis involves  
258 elaborate molecular mechanisms that have evolved to defend genomes against TEs in  
259 both plants and animals [43, 45, 46], involving small interfering RNAs that are  
260 produced in response to the presence of TEs in the genome. The great diversity of  
261 sequences and genomic locations in which they can be inserted means that the  
262 mobility of TEs is their only common distinguishing feature; this is their ‘Achilles’  
263 heel’ that allows cells to detect them [46].

264         In animals, the RNAs involved in TE silencing belong to a class called  
265 piRNAs. In *Drosophila*, maternal TE-derived piRNAs are incorporated into the egg  
266 before fertilization, resulting in a form of epigenetic inheritance. However, the  
267 maintenance of effective TE suppression requires the presence in the DNA of  
268 genomic clusters of TE insertions, providing a ‘memory’ of previously active  
269 elements, like the immune memory systems that defend cells against previously  
270 encountered pathogens. Once acquired, these clusters of TE-derived sequences prime  
271 the resistance pathways anew each generation through a self-perpetuating  
272 amplification process called ‘ping-pong’, whereby the piRNAs produced by the  
273 clusters interact with those from active TEs to repress transposition [47, 48]. When  
274 maternally-derived piRNAs from TEs are not generated, there may be insufficient  
275 piRNA for repression, explaining the maternal inheritance associated with hybrid  
276 dysgenesis.

277         This intricate system is a biological marvel, which represents the outcome of  
278 natural selection to overcome the harmful effects of TE mobilization. Hybrid  
279 dysgenesis is simply a product of the temporary failure of this system; it is a transient,  
280 pathological phenomenon, and occurs in nature only when a new TE type is

281 introduced into a population, as is currently happening with the *P* element in *D.*  
282 *simulans* [43].

283         The non-nuclear transmission of small interfering RNAs provides, however, a  
284 potential mechanism for the inheritance of an adaptively useful trait acquired in  
285 response to an environmental treatment [47]. An example has been described in  
286 *Caenorhabditis elegans*, where small interfering RNAs derived from an RNA virus,  
287 conferring protection against infection, can be transmitted through the cytoplasm over  
288 several generations of self-fertilisation [49]. It remains to be determined how  
289 frequently such processes occur in nature.

290

### 291 **(b) Paramutation**

292 Another exception to Mendelian inheritance is paramutation [50, 51], whose  
293 discovery in maize involved puzzling interactions between two alleles at a single  
294 locus, in which a paramutagenic allele induced a heritable change in the expression of  
295 another (paramutable) allele, without changing its DNA sequence; the paramutated  
296 allele may itself become paramutagenic. Although paramutation looks like a form of  
297 directed mutation (see below), and the paramutated state can persist for many  
298 generations, the change is usually impermanent, decaying over time. Paramutation is  
299 now known to occur in fungi, animals and plants [51].

300         Genetic analyses have revealed that paramutation has similarities with  
301 silencing of transposons by small RNAs. Reactivation of an inactive piRNA-  
302 producing cluster in *Drosophila* can be induced by interactions with a different, but  
303 partially homologous, cluster within a genome to produce active, paramutated  
304 versions that can silence new TE sequences that insert into old or new clusters [51,  
305 52]. This may explain the progressive establishment over several generations of  
306 repressive capacity after hybrid dysgenesis-producing *I*- or *P*-elements are introduced  
307 by paternal inheritance into a cytoplasm without *I*- or *P*-homologous piRNAs [52].  
308 There is no firm evidence as yet that paramutation plays a role in adaptive evolution,  
309 although it could act like a type of meiotic drive [53], with the paramutated allele  
310 increasing in frequency in the population by propagating new copies of itself at the  
311 expense of alternative alleles. Rather, it appears to reflect a process that evolved in  
312 response to threats to genome integrity, and is strongly associated with the presence of  
313 repetitive DNA sequences [51].

314

315 **(c) Stability of transmission of epigenetic marks across generations**

316 Epigenetic marks such as DNA or histone methylation can undoubtedly be  
317 transmitted across cell divisions in unicellular organisms. Early in the history of  
318 genetics, it was recognised that transmission across cell divisions of phenotypic  
319 changes induced by environmental conditions could occur in protists, but tended to  
320 revert after several divisions. The best-studied example of such *Dauermodifikationen*  
321 [54] is serotype switching in *Paramecium*, in which temperature can affect which  
322 gene is expressed out of a large set that control surface antigens [55]. The functional  
323 significance of this plastic response is still unclear.

324 In multicellular organisms, the role of epigenetic chromatin modification in  
325 stable cell differentiation during multicellular development is also, of course, well  
326 established [26]. The crucial question for evolutionary biology is how often such  
327 marks are transmitted between generations via sexual reproduction, independently of  
328 any causal DNA sequence differences. For the development of a fertilised egg into an  
329 adult, it is important for the zygote to be totipotent, suggesting that epigenetic marks  
330 affecting gene regulation should normally be erased during germ cell production. This  
331 is indeed usually the case in animals, apart from some exceptions such as imprinted  
332 genes in mammals, where either paternally- or maternally-derived genes are inactive  
333 [26, 35, 39]. The most convincing cases of trans-generational inheritance of  
334 epigenetic marks in animals are associated with repetitive sequences, and it has been  
335 proposed that selection in favour of mechanisms that maintain repression of their  
336 expression has been responsible for the ability to transmit these marks across  
337 generations [56].

338 In plants, however, resetting of epigenetic marks such as methylation is less  
339 efficient than in animals, and there is evidence from crossing experiments for  
340 transmission of methylation states across generations [57] especially methylation of C  
341 at CpG dinucleotide sites [57-59]. The methylation status of such C sites is, however,  
342 quite unstable, with a higher frequency of losses than gains, and overall ‘mutation’  
343 rates around  $10^{-4}$  per basepair per generation, 5,000 times higher than those for DNA  
344 nucleotide changes. Despite this instability, such epiallelic variants could have a role  
345 in evolution [58]: with reversion at a rate of  $10^{-4}$ , a selective advantage of 1% in  
346 heterozygotes would allow an advantageous epiallele to spread to an equilibrium  
347 frequency of 99% [60]. However, mutations to deleterious alleles create a genetic

348 load. In large populations, the load depends strongly on the mutation rate [60]. If CG  
349 dinucleotide methylation were often functionally significant, such a load would select  
350 for a lower epimutation rate [61]. The high rate that is observed thus suggests that the  
351 sites involved are mostly irrelevant to fitness. Indeed, a recent population study  
352 capable of detecting very weak selection suggests that CG epimutations outside TE  
353 insertions are close to neutral, and thus probably not relevant to adaptive evolution  
354 [59].

355

#### 356 **(d) Contributions of epiallelic variation to discrete trait variation**

357 While many major mutations have been found to be associated with DNA sequence  
358 changes and TE insertions, there is little evidence that stable epiallelic variants  
359 without associated DNA sequence variants are abundant among spontaneous  
360 mutations. A much-cited exception is the peloric flower phenotype in the toadflax  
361 *Linaria*, which appears to arise frequently despite causing almost complete sterility of  
362 the affected flowers [62]. RNA expression of the gene involved, *cycloidea*, is  
363 completely silenced in peloric flowers, due to hypermethylation. However, silencing  
364 maps to a single nucleotide polymorphism in an unmethylated region 308 basepairs  
365 downstream of the stop codon [63]. It affects only the rarer *cyc308G* allele, and not  
366 the *CYC308A* allele. Silencing is recessive, and all plants with peloric flowers are GG  
367 homozygotes, with both copies silenced. This genotype also often has wild-type  
368 flowers, and the degree of *cycloidea* methylation correlates with the strength of the  
369 phenotypic effect. This demonstrates epigenetic control of peloric flowers, with  
370 incomplete penetrance, when the DNA sequence variant is present. There is no  
371 evidence that peloric mutations are evoked by environmental challenges, contrary  
372 what is sometimes claimed [21]. Some other examples of epiallelic mutant  
373 phenotypes in plants are described in [57].

374

#### 375 **(e) Contributions of epiallelic variation to quantitative trait variation**

376 If epialleles were to contribute to variability in a trait subject to stabilizing selection,  
377 standard evolutionary models of the interaction between stabilizing selection and  
378 mutation [64] imply that the high epiallelic mutation rate mentioned above could  
379 potentially contribute substantially to genetic variance, and hence to responses to  
380 selection if the phenotypic optimum changes. The numerous measurements of both  
381 mutational and standing variability in quantitative traits [64, 65] include any potential

382 contributions from epiallelic variants. Finding that epigenetic variation plays a  
383 significant role in quantitative trait variability would thus not radically change our  
384 understanding of how populations respond to selection.

385         Nonetheless, the question of the extent to which epiallelic variants contribute  
386 to natural quantitative trait variability is of great interest, where critical evidence is  
387 currently lacking. Experiments using a strain of *A. thaliana* that had been stripped of  
388 its methylation, and then allowed to remethylate, suggest that variability in  
389 methylation amongst genetically identical progeny is associated with heritable  
390 variability in quantitative traits [57]. This shows that quantitative traits can be affected  
391 by epiallelic variability. However, it remains unclear to what extent natural trait  
392 variation is caused in this way. For one trait, gene expression levels in *A. thaliana*, the  
393 contribution of epialleles has been estimated [66]. In this highly self-fertilising plant,  
394 populations are strongly spatially isolated. DNA methylation variants are therefore  
395 correlated with sequence variants in the DNA, complicating the analyses. Indeed,  
396 genome-wide differences in SNPs can explain the overall expression results just as  
397 well as DNA methylation differences, and *vice versa*. To take population structure  
398 into account, genome-wide association (GWAS) analyses were done using SNP-based  
399 kinship estimates. For *cis*-acting methylation variants (the majority of the effects  
400 detected), only 63 significant methylation associations were found without an  
401 accompanying SNP association. Thus, fewer epigenetic loci appear to affect gene  
402 expression than SNPs; their effects are also smaller than those of SNPs. Of course,  
403 there may be detection biases against methylation variants that are not associated with  
404 SNPs at the sites in question, and further research is clearly desirable.

405

406 **(f) Does epigenetic inheritance contribute to the transmission of**  
407 **adaptive acquired characters?**

408 If epigenetic changes producing *adaptive* changes in phenotypes induced by external  
409 circumstances were often transmitted to the offspring, this would involve a major  
410 change in outlook. The so-called ‘Central Dogma’ of molecular biology, e.g. Chap. 4  
411 in [67], states that information flows from nucleic acid sequence to protein sequence,  
412 and not vice versa. More generally, there is no known mechanism for systematically  
413 generating adaptive and heritable DNA sequence variation (see the discussion of  
414 ‘directed mutation’ in section 5 below).

415         As described above, mechanisms have evolved by which specific kinds of

416 adaptive responses can potentially be transmitted across one or more generations,  
417 involving epigenetic marks or the production of small RNA molecules that are  
418 transmitted through the germ cells. If these changes could produce stable adaptive  
419 traits in the offspring, and if they occurred sufficiently frequently, such ‘Lamarckian’  
420 inheritance could play a significant role in phenotypic variation and evolution [19,  
421 21]. However, as noted long ago by Haldane [5] and Muller [27], such a process is  
422 unlikely to be of general importance, because a large body of genetic experiments has  
423 established the ineffectiveness of selection on homozygous lines, which lack genetic  
424 variation but still show phenotypic variation. In striking contrast, family selection,  
425 with no exposure of the selected individuals to the environment in which the trait is  
426 favoured, is highly effective [68]. One of the most spectacular examples of non-  
427 genetic phenotypic differences is provided by the sterile worker castes of social  
428 insects. Darwin himself pointed out that these could not possibly have evolved by a  
429 Lamarckian mechanism, but must be the product of selection on the genotypes of the  
430 reproductive individuals to produce workers with phenotypes adapted to different  
431 tasks [68]. There is therefore a long-standing and strong empirical basis for rejecting  
432 the inheritance of acquired characters as a frequent phenomenon (see also the  
433 discussion of directed mutation in section 5).

434         Epigenetic marks certainly change in response to environmental factors, e.g.  
435 vernalisation in flowering plants [69]. However, when consistent epigenetic changes  
436 are seen in response to specific treatments or environments, transmission to the next  
437 generation is rarely tested, and it is often not known whether these change any  
438 phenotype or affect gene expression [70, 71]. A thorough review of the evidence on  
439 mammals concluded that evidence for “*widespread transgenerational epigenetic*  
440 *inheritance is lacking to date*”, and that “*the concept of transgenerational epigenetic*  
441 *inheritance in humans remains equivocal*” [39].

442         A convincing, but artificial, case has been described in *C. elegans*, in which  
443 heat-induced expression of a multicopy array of the gene coding for the heat-shock  
444 protein Hsp90 was transmitted for 14 generations, through both eggs and sperm, due  
445 to loss of histone HK3K9 methylation from the array [72]. No such transmission was,  
446 however, found with the normal situation of a single copy of the gene. Statistical  
447 concerns have been raised about many other published claims of multigeneration  
448 transmission of acquired traits [73, 74]. Overall, the evidence that such transmission is  
449 a common phenomenon is weak [75], even in plants where the germline is not sharply

450 distinct from the soma [57, 76].

451 Another situation that has been claimed to involve the inheritance of acquired  
452 characters [20] involves the Clustered Regularly Interspaced Short Palindromic  
453 Repeats (CRISPR) defence mechanism that protects prokaryote genomes from  
454 transmissible genetic elements such as bacteriophages and conjugative plasmids.  
455 These systems have similarities to the defences against TEs described above, in that  
456 'naïve' cells acquire the ability to recognise new infections. Again, this represents a  
457 change elicited by a specific environmental factor (invasion), which is heritable by a  
458 cell's descendants (a 'mutation'). In these systems, short pieces of foreign DNAs that  
459 enter a cell are cut out at 2-5 bp sequence motifs (called "Protospacer Adjacent  
460 Motifs" or PAMs) and integrated into a repeat-containing CRISPR locus in the host  
461 cell, which thus becomes interleaved with 'spacer' sequences that match specific  
462 sequences of foreign origin [77]. These sequences provide a 'memory' of foreign  
463 sequences that the cell has received. Complementarity between CRISPR-expressed  
464 RNAs and sequence in invading DNA ('proto-spacer' sequence) allows cells to detect  
465 the corresponding sequence (e.g. phage) during subsequent infections, and target it for  
466 destruction, similarly to the RNA interference mechanism that inhibits gene  
467 expression in eukaryotes [1, 77].

468 Importantly, however, the system includes no function to ensure that the  
469 'mutations' (new spacers in a CRISPR array) benefit the cell, rather than harming it.  
470 Elements with the required sequence signatures can generate the targeting outcome,  
471 whether or not they target a sequence that forms part of something that is harmful to  
472 the cell. Indeed, a plasmid carrying a gene whose loss reduces cells survival can be  
473 destroyed. Some spacers target the cells' own DNA, which is clearly maladaptive and  
474 can cause cell death. This system, like other mutational processes, generates  
475 mutations irrespective of their benefits, and cell lineages that are lucky enough to gain  
476 suitable spacers will tend to increase, while ones that produce damaging ones, or cell  
477 death, are eliminated [1].

478

### 479 **(g) Lateral gene transfer**

480 A substantial proportion of some prokaryotes' genomes can consist of horizontally  
481 acquired sequences, whereas horizontal transmission appears to be much less  
482 prevalent in eukaryotes [78]. The acquired sequences may sometimes be adaptive in



483 their new organismal environment, but need not be. In any organism where such gene  
484 transfers may occur, a gene-centred perspective is necessary, in which the genes (or  
485 sequences) are the replicators that are subject to natural selection, and other  
486 components of the genome are part of their environment. The acquisition of  
487 selectively favourable DNA sequences by lateral gene transfer in prokaryotes is thus  
488 entirely consistent with neo-Darwinism [1], and labelling it as ‘quasi-Lamarckian’  
489 [20] is misleading.

490

#### 491 **4. Sequence versus epigenetic changes in phenotypic evolution**

492

493 Modern molecular genetic methods allow evolutionary biologists to detect selection  
494 from DNA sequence data. Many such studies have directly detected selection acting  
495 on DNA sequence variants in either protein sequences or regulatory non-coding  
496 sequences, using analyses of substitutions along evolutionary lineages [79],  
497 polymorphisms within natural populations [24], or a combination of the two [23]. In  
498 many cases, however, the basis for inferring selection is indirect, often coming simply  
499 from a ‘footprint of selection’ such as an observation of reduced variability in a small  
500 region of the genome [24, 25], suggesting that the spread of an initially rare variant (at  
501 an unknown selected site) has caused the ‘hitchhiking’ of variants at closely linked  
502 neutral or nearly neutral variants. In such cases, the selected variant could be either a  
503 DNA sequence variant or an epiallele.

504

##### 505 **(a) The causes of new mutations**

506 At least two approaches can help to test the extent to which DNA sequence versus  
507 epigenetic variants contribute to adaptive evolution. First, one can assess the  
508 contributions of different types of variants to components of *de novo* mutational  
509 variation in traits of potential evolutionary significance. Innumerable molecular  
510 genetic analyses have shown that new mutations with detectable phenotypic effects,  
511 tabulated in databases such as *OMIM* (mutations causing human genetic diseases),  
512 *Flybase* and *Wormbook*, frequently involve DNA sequence changes. There may,  
513 however, be a bias towards detecting sequence changes, due to the difficulty of  
514 characterising epigenetic changes.

515         Systematic, unbiased surveys of the causes of mutations causing specific  
516 phenotypes are currently scarce, because such work became technically possible only

517 recently. However, an analysis of mutations that suppress the harmful fitness effects  
518 of 251 deletion mutations in yeast genes identified sequence mutations in 86% of  
519 cases; as the effects of some sequence mutations must have been undetectable (false  
520 negatives), this leaves little scope for epigenetic variants [80]. A screen of exome  
521 sequences of 4,923 human families ascertained through an offspring with a severe  
522 developmental disorder detected coding sequence mutations in 42% of cases [80].  
523 This study was not designed to detect either regulatory mutations in non-coding  
524 sequence or major chromosomal rearrangements, two further important sources of  
525 harmful mutations, so that there is probably only a narrow margin for epigenetic  
526 variants.

527

### 528 **(b) The causes of phenotypic variants**

529 An approach that is more directly relevant to evolution is to assess the extent to which  
530 epigenetic versus genetic variants have caused phenotypes involved in putatively  
531 adaptive phenotypic change or variation. Martin and Orgogozo [81] tabulated 252  
532 examples of phenotypic differences within natural populations, or between closely  
533 related species, where linkage mapping localized genetic factors to a small region;  
534 245 further examples involve domesticated animals or plants. Only one of the natural  
535 cases is a potentially epigenetic variant, the *Drosophila* zygotic lethal male rescue  
536 factor, a change associated with repetitive DNA in heterochromatin (this compilation  
537 also included the *Linaria* peloric mutation; however, as discussed above, this is  
538 associated with a sequence change). In 184 cases of natural phenotype differences,  
539 associated DNA sequence variants were found, while in 67 (26.6% of the total) no  
540 associations of any kind were detected. In many of the cases where sequence  
541 differences were detected, these were nonsynonymous mutations or  
542 insertions/deletions in coding sequences. Such variants are usually kept at low  
543 frequencies by selection; they are thus plausible candidates for causing the phenotypic  
544 differences, as it is unlikely that they could hitchhike to high frequencies along with  
545 an advantageous epiallele.

546 Ideally, manipulation of DNA in transgenic experiments, where epigenetic  
547 marks are necessarily removed, should be used to determine whether candidate causal  
548 sequence variants have functionally relevant effects. Such tests are possible only for  
549 variants with large phenotypic effects, but provide a guide to what is likely to be the  
550 case more generally. A pioneering study of this kind examined the *Alcohol*

551 *dehydrogenase (Adh)* electrophoretic polymorphism of *D. melanogaster*, where fast  
552 electrophoretic alleles are associated with higher ADH protein production than slow  
553 alleles. This difference was mainly due to an insertion of several base pairs in the first  
554 intron of the fast allele, together with several other regulatory sequence variants [82].  
555 Stern and Orgogozo [83] listed 46 successful functional studies among their  
556 ‘restricted’ dataset of 162 phenotypic differences associated with DNA sequence  
557 differences. Given the technical difficulties of this type of experiment, this is an  
558 impressive rate of success. A more recent survey of this kind [81] did not record  
559 transgenic experiments; however, none out of 100 later papers that cited it indicated  
560 any role for epigenetic variants. Nine of these described transgenic experiments, all of  
561 which identified sequence changes that caused naturally occurring phenotypic  
562 differences in yeast, plants, and animals.

563         With the increasing use of CRISPR technology for genetic manipulation, we  
564 anticipate a rapid increase in such tests. Strategies for extending these approaches to  
565 differences among taxa that cannot interbreed, and hence are inaccessible to genetic  
566 mapping, are also being developed. A notable example is the analysis of the effect of  
567 the *Fzd8* enhancer in promoting larger brain size in humans compared with  
568 chimpanzees [84]. This enhancer was identified as a candidate by screening  
569 noncoding sequences that have enhancer roles in neocortex development, and were  
570 highly conserved in most mammals but evolved rapidly in the human lineage.  
571 Transgenic experiments in mice revealed that the human enhancer sequence caused  
572 larger brain size than the chimpanzee sequence.

573

### 574 **(c) Some general implications**

575 Genetic studies of adaptive phenotypes have yielded several further important  
576 conclusions. First, there are now many examples of phenotypic differences within and  
577 between species whose genetic control maps to a small region, but with multiple  
578 nucleotide differences within the region being causally involved [85]. This supports  
579 Darwin’s and Fisher’s view that adaptive phenotypes are usually built up by a series  
580 of relatively small changes, which has been challenged by proponents of the EES [15,  
581 19].

582         Second, phenotypes that show plastic responses to environmental conditions  
583 also often show considerable genetic variation in these responses, and DNA sequence  
584 variants associated with these heritable differences have been identified, supporting

585 the view that plasticity has evolved in a neo-Darwinian fashion [4]. For example,  
586 vernalisation responses in flowering plants involve a period of exposure to cold that is  
587 required for seed germination. (This was the basis for the notorious Lamarckian  
588 theories of T.D. Lysenko, which seriously damaged Soviet agriculture [10, 11]).  
589 Vernalisation is under the control of a complex epigenetic regulatory system, which is  
590 reset each generation [57, 69]. Natural vernalisation response differences are  
591 controlled by DNA sequence variation in cis-acting regulatory sequences [86, 87].

592 In contrast to the rigorous empirical evidence for the role of DNA sequence  
593 variants in adaptive evolution that we have outlined, there is currently little evidence  
594 for effects of epigenetic changes, although more data are required. Recent claims for  
595 such effects have been based on evidence that changes affecting the methylome are  
596 more numerous than some types of sequence variants in evolving lineages of  
597 Darwin's finches [88] and darter fish [89]. Such comparisons, however, provide no  
598 evidence that the epigenetic variants in question had any role in phenotypic evolution.

599 Several theoretical studies show that the general framework of population and  
600 quantitative genetics applies to epigenetic inheritance [90, 91]; indeed, the basic  
601 theory was developed half a century before the molecular basis of inheritance was  
602 determined. Combining modes of inheritance that differ in their mutation rates and  
603 transmission patterns can alter the outcome of selection in complex ways – similar to  
604 the complexities possible with maternal effects on quantitative traits mentioned in  
605 section 3.e [40, 41]. However, this is not of fundamental significance as far as the  
606 general properties of evolutionary dynamics are concerned. Even if new alleles  
607 affecting a trait are induced by a specific environment, they can contribute to  
608 adaptation only if transmission is fairly stable and the environment is quite  
609 predictable, so that the new allele remains advantageous in future environments [92,  
610 93].

611 Finally, we note that demonstrating a causal role for epialleles in an adaptive  
612 phenotype is a necessary, but not sufficient, condition for radical changes to the neo-  
613 Darwinian theory of adaptive evolution. To support a neo-Lamarckian mode of  
614 evolution, evidence would be needed that (i) a given environmental treatment tends  
615 systematically to induce heritable, adaptive epiallelic variants (ii) natural selection is  
616 not involved in the spread of such variants through populations (iii) the variants in  
617 question can be stably transmitted for many generations in the absence of the  
618 treatment. If the claim is instead that variation is systematically biased towards

619 generation of adaptive variants, which are then picked up by selection, then one has to  
620 show that this bias has a significant effect on the outcome, beyond what would have  
621 been produced by selection on random variation. In view of the vast body of evidence  
622 for neo-Darwinian mechanisms, the principle that ‘extraordinary claims require  
623 extraordinary evidence’ [12, 94] implies that such stringent criteria must be met  
624 before we should consider abandoning or substantially modifying neo-Darwinism.  
625 The case of ‘directed mutation’ that we discuss next brings out the importance of  
626 experimental rigour in dealing with these problems.

627

## 628 **5. Directed mutation**

629 The concept of ‘directed mutation’ proposes that organisms might respond to an  
630 environmental challenge by an increased mutation rate in a target DNA sequence that  
631 specifically results in mutants with higher fitness in the new environment [95]. This  
632 concept is similar to the inheritance of acquired characters, but differs from it because  
633 it involves changes in the genetic material without a prior change in the phenotype. It  
634 traces its origin back to studies of rapid adaptive responses by bacteria to new  
635 laboratory environments, which revealed astonishing speeds of bacterial adaptation.  
636 For example, naturally occurring *lac*<sup>-</sup> strains of *Escherichia coli*, known as *E. coli*  
637 *mutabile*, are normally unable to ferment lactose, but can acquire the ability to do so a  
638 day or two after transfer to lactose as a carbon source [96], and maintain it when  
639 grown in a lactose-free medium.

640       Until the 1940s, it was widely believed that exposure to the new environment  
641 directly induced these adaptive, heritable changes, and bacteriology was “the last  
642 stronghold of Lamarckism” [97]. But this ended when bacterial inheritance became  
643 understood. Brilliant genetic and biochemical studies developed and verified a  
644 straightforward, neo-Darwinian interpretation for these observations [88]: if rare  
645 mutations producing the adaptive phenotype constantly arise independently of the  
646 state of the environment, they would have a selective advantage and quickly replace  
647 their less fit competitors when grown in the new environment [28]. The vast numbers  
648 of cells in bacterial cultures, and the short times between cell divisions in cultures of  
649 dividing cells, make this inevitable. The Lamarckian alternative hypothesis can be  
650 tested by asking whether the mutant bacteria are already present in the population  
651 *before* exposure to the selective agent (which then merely reveals their presence —  
652 the neo-Darwinian interpretation). Several experimental tests were devised, starting

653 with the ‘fluctuation test’ [98]. By the mid-1950s, the evidence overwhelmingly  
654 supported the neo-Darwinian interpretation.

655         The universality of this conclusion was later challenged by results from  
656 bacteria and yeast [95, 99]. However, as reviewed by Maisnier-Patin and Roth [99], a  
657 neo-Darwinian explanation exists for findings that apparently suggested the  
658 involvement of mutations that specifically conferred an adaptive phenotype.  
659 Experiments involving *E. coli* with leaky mutations in a *lac* operon gene found that  
660 growth on medium with lactose as the carbon source is severely impaired, but that,  
661 over time, colonies appeared, indicating that growth was occurring. Moreover,  
662 mutants conferring the ability to grow on lactose appeared only in the presence of  
663 lactose [95, 99]. Inability to grow on lactose is due to a frameshift mutation in the  
664 *lacZ* member of the *lac* operon carried on a plasmid present in low copy number. 90%  
665 of revertants regaining the ability to grow on lactose had a stable compensating  
666 mutation in the *lacZ* gene, while 10% had unstable tandemly amplified copies of the  
667 mutant gene. About 100 times more mutations occurred than would be expected based  
668 on mutation rates under non-selective conditions. 10% showed a 100-fold increase in  
669 the mutation rate, affecting all genes tested, probably attributable to the stressful  
670 conditions experienced by the bacteria. But the critical question is: what is the source  
671 of the 90% of revertants with no increased mutation rate? These appear to be targeted at  
672 the *lacZ* gene to specifically produce beneficial revertants.

673         It turns out that the observations do not require directed mutations, and that a  
674 neo-Darwinian explanation is more likely, once the intricate experiments are  
675 understood in detail [99]. This explanation proposes that spontaneous fluctuations  
676 sometimes produce cells with increased numbers of the plasmid carrying the (mutant)  
677 *lacZ* gene. This would allow a non-dividing cell to use lactose to provide sufficient  
678 energy to copy the plasmids, increasing the probability of occurrence of *lac*<sup>+</sup>  
679 revertants, which then permit the cell to divide. Descendant cells’ plasmids carry  
680 revertant genes, making it appear that mutations were targeted to the site involved in  
681 the reversion. Having multiple copies of the plasmid may also increase the mutation  
682 rate, because the plasmid carries an error-prone DNA polymerase gene. Natural  
683 selection can thus produce the appearance of directed mutagenesis. This model, while  
684 not fully confirmed experimentally, is consistent with all currently available data. As  
685 Maisnier-Patin and Roth [99] comment “*it is important to remember that natural*  
686 *selection sees almost everything and is always watching*”.

687

688 **6. Is there an evolvability problem?**

689

690 **a) Genetic variation and evolvability**

691 It is sometimes stated that standard modes of generating mutational variability are  
692 inadequate to explain the speed of adaptive evolution, and that additional processes  
693 are thus needed to ensure the ‘evolvability’ of a species, a concept discussed from a  
694 neo-Darwinian perspective in [100]. For example, Laland *et al.* [14] state that  
695 “*Inclusive models help to explain a wide range of puzzling phenomena, such as the*  
696 *rapid colonization of North America by the house finch, the adaptive potential of*  
697 *invasive plants with low genetic diversity, and how reproductive isolation is*  
698 *established*”. However, a vast literature on artificial selection [65] and experimental  
699 evolution [101] shows that selection can change almost any trait over a very short  
700 timescale, implying that there is usually ample heritable variation on which selection  
701 can act. As Darwin emphasised in Chapter 1 of *The Origin of Species* [102], examples  
702 such as dogs and domestic pigeons demonstrate the power of artificial selection to  
703 alter phenotypes, often resulting in changes as great as those distinguishing different  
704 genera.

705         These observations provide strong evidence that selection can quickly take a  
706 population towards a nearby fitness optimum, without any need for special  
707 mechanisms generating new variability. Even in humans, with their relatively small  
708 population size over most of our history, the mutation to sickle-cell haemoglobin that  
709 confers resistance to malaria has spread independently at least four times, in different  
710 populations, and hundreds of other polymorphisms for mutations conferring malaria  
711 resistance are known [103]. Rates of long-term evolution are thus probably largely  
712 controlled by environmental changes, and not by the supply of mutations. This  
713 conclusion was reached by the founders of the MS, and many recent studies support it  
714 [104].

715         However, some situations involve evolution to new ‘adaptive peaks’ that can  
716 only be reached by crossing a ‘valley’ of phenotypes with reduced fitness, especially  
717 when a coordinated complex of characters changes. Goldschmidt suggested that such  
718 phenotypic changes require complex macromutations, which, in a single step, produce  
719 beneficial multi-trait combinations [9]. This proposal has been thoroughly tested by  
720 genetic analyses in the case of mimicry, and rejected in favour of the process of

721 stepwise improvement proposed by Fisher [8], whereby a mutation with a relatively  
722 large effect on one aspect of mimetic resemblance produces an adequate, but  
723 imperfect, mimic, with the subsequent accumulation of more minor changes that  
724 improve mimicry [105, 106, Chap.3]. While mutations with major effects on  
725 individual traits can certainly contribute to adaptive evolution (see section 4 above),  
726 as was well-known to the founders of the MS [5], there is no evidence for a role for  
727 macromutations of the type postulated by Goldschmidt and his followers [3].

728 As we have seen, however, critics of neo-Darwinism often argue that more  
729 attention should be paid to the availability of adaptive variation. If we discard the  
730 possibility that induced adaptive variability is at all common, as argued above, there  
731 are only two well-established processes whose rates of occurrence significantly affect  
732 the amount of variability available for adaptive evolution – mutation and genetic  
733 recombination. Analysing the evolution of these genome properties has been central  
734 in evolutionary biology, starting with work by Fisher at the beginning of the MS [8].  
735

#### 736 **b) The evolution of mutation rates, sex and genetic recombination**

737 Selection on variants that alter the mutation rate has been intensively studied, both  
738 theoretically and experimentally [61, 107, 108], with the aim of understanding the  
739 outcome of the conflict between the potential advantage of producing beneficial  
740 mutations, and the fact that most mutations that affect fitness are deleterious [27, 61].  
741 In largely asexually reproducing populations, an allele that causes an increased  
742 mutation rate (a ‘mutator’) can remain linked to any beneficial mutations that it  
743 induces, and hence increase in frequency by ‘hitchhiking’ [100]. Adaptation in  
744 microbial populations indeed often leads to evolution of mutator strains whose DNA  
745 repair is defective, and which produce beneficial mutations more frequently than non-  
746 mutators, resulting (often temporarily) in an increased mutation rate [107] In sexual  
747 populations, however, recombination quickly disassociates mutator alleles from any  
748 beneficial mutations, and their increased frequency of deleterious mutations favours  
749 alleles conferring lower mutation rates [61, 108].

750 The elaborate molecular machinery for correcting errors in DNA replication  
751 strongly suggests that natural selection has generally favoured reduced mutation rates  
752 [61]. However, there are examples where special mechanisms have evolved to  
753 generate variability in situations where there is intense selection for rapid change, as  
754 in pathogenic microbes whose surface antigens are targeted by the host immune



755 system [100]. A particularly well-studied example is the ‘cassette’ of *vlsE* genes of  
756 the Lyme disease bacterium *Borrelia burgdorferi*, in which there is a group of similar  
757 but diverse genes that code for the VlsE antigen, only one of which is expressed at a  
758 given time by virtue of its presence at an expression site [109]. Recombination with  
759 this site produces expression of different versions of the antigen, and selection favours  
760 sequence differences in members of the cassette, partly because of mutation-prone  
761 sequences in regions targeted by host antibodies [109].

762 Work on the evolution of sex and recombination over many decades has built  
763 a sophisticated theoretical understanding of how selection acts on genetic variants that  
764 modify the rate of genetic recombination or the frequency of sexual reproduction, as  
765 described in [106, Chap.3] and [110]. One important conclusion is that genetic  
766 recombination can be favoured because it facilitates responses to selection by  
767 generating new combinations of favourable alleles, and the frequencies of sex [111]  
768 and recombination [112] indeed tend to increase in experimentally selected  
769 populations. Crucially, studies of both mutation and recombination show that,  
770 although selection may lead to the adaptive modulation of the *amount* of variation,  
771 there is no *bias* towards the production of beneficial variants.

772

### 773 **c) Canalisation and robustness**

774 While much more empirical work remains to be done, the research just outlined  
775 shows how features of the genome that affect evolvability can be understood using the  
776 principles of the MS. Similar arguments apply to the ‘canalisation’ of developmental  
777 systems, which buffers them against genetic or environmental perturbations that  
778 produce deleterious phenotypes, leading to phenotypic ‘robustness’ [113]. For  
779 example, the Hsp90 heat shock protein is a ‘chaperone’ that minimises deleterious  
780 protein misfolding. When this system is disrupted, phenotypic variants are revealed.  
781 Because these might occasionally be beneficial, it has been suggested that Hsp90 is an  
782 ‘evolutionary capacitor’ that evolved *because* its disruption in challenging  
783 environments occasionally reveals useful heritable variants [114]. However, systems  
784 such as Hsp90 are more likely to have evolved to *minimise* deleterious phenotypic  
785 variation; their breakdown is probably maladaptive, occurring when stress impairs  
786 normal control systems [113].

787 The existence of these buffering mechanisms contradicts claims that  
788 “*Developmental systems facilitate well-integrated, functional phenotypic responses to*

789 *mutation or environmental induction*” (point (iii) of Table 1 in [15]), as does the  
790 overwhelming evidence that most mutations with noticeable phenotypic effects are  
791 deleterious [27]. While there are unquestionably many examples of adaptive  
792 phenotypic plasticity, there are strong reasons for thinking that these are *evolved*  
793 responses to environmental challenges, consistent with the evidence for genetic  
794 variation in plasticity described in section 4.c, rather than inherent properties of  
795 developmental systems [3, 4]. This also applies to cases where a plastic response can  
796 be transmitted over one or more generations [35, 36].

797

## 798 **7. Conclusions**

799 We have focussed our discussion on the sources of the variability used in *adaptive*  
800 evolution. However, it is important to understand that contemporary evolutionary  
801 biology does not take a dogmatically adaptationist or pan-selectionist view of the  
802 evolutionary causes of all characteristics of living organisms. This is especially true  
803 for properties of the genome itself, many of which must involve interactions between  
804 the effects of mutational processes, selection and genetic drift. Some examples are  
805 reviewed in [115] and Chap. 10 in [106]. For example, the effectiveness of selection  
806 is greatly weakened when genetic recombination is very infrequent, which explains  
807 the evolutionary degeneration of Y chromosomes through the accumulation of  
808 deleterious mutations (despite the fact that the suppression of crossing over between  
809 the ancestors of X and Y chromosomes was originally favoured by selection).  
810 Furthermore, selfish genetic elements such as TEs and segregation distorters can  
811 promote their own spread within genomes and populations at the expense of the  
812 fitness of their hosts [53]. Nevertheless, we finish by re-emphasising the central  
813 concept of neo-Darwinism and the MS: allele frequency change caused by natural  
814 selection is the only credible process underlying the evolution of adaptive organismal  
815 traits.

816

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820

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822

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