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REVIEW

Evolution of adaptive phenotypic traits without positive Darwinian selection

AL Hughes

Recent evidence suggests the frequent occurrence of a simple non-Darwinian (but non-Lamarckian) model for the evolution of adaptive phenotypic traits, here entitled the plasticity–relaxation–mutation (PRM) mechanism. This mechanism involves ancestral phenotypic plasticity followed by specialization in one alternative environment and thus the permanent expression of one alternative phenotype. Once this specialization occurs, purifying selection on the molecular basis of other phenotypes is relaxed. Finally, mutations that permanently eliminate the pathways leading to alternative phenotypes can be fixed by genetic drift. Although the generality of the PRM mechanism is at present unknown, I discuss evidence for its widespread occurrence, including the prevalence of exaptations in evolution, evidence that phenotypic plasticity has preceded adaptation in a number of taxa and evidence that adaptive traits have resulted from loss of alternative developmental pathways. The PRM mechanism can easily explain cases of explosive adaptive radiation, as well as recently reported cases of apparent adaptive evolution over ecological time.
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'Je n'ai pas eu besoin de cette hypothèse.'
–Pierre-Simon de Laplace

Although Darwin (1859) entitled his great work *The Origin of Species*, he is best known for proposing a mechanism to account for the origin of adaptations. This mechanism, as reinterpreted by Neo-Darwinism in the light of Mendelian genetics, can be summarized as follows: following an environmental change, an allelic variant, which either has arisen as a new mutation or which had already existed in the population at a low frequency, increases in frequency and eventually reaches fixation because of the fitness advantage it confers in the new environment (Fisher, 1930). I will refer to this mechanism as the Neo-Darwinian mechanism; and, following general usage, I will refer to an allele that has been fixed by this process as one that has been fixed by positive Darwinian selection. The Neo-Darwinian mechanism is often assumed by biologists to be the only source of adaptive traits of organisms, to the point where 'adaptive evolution' and 'positive (Darwinian) selection' are treated as interchangeable terms in the literature.

In developing his neutral theory of molecular evolution, Kimura (1983) argued that most evolutionary change at the molecular level occurs as the result of the fixation by genetic drift of selectively neutral or nearly neutral mutations. Nonetheless, Kimura frequently acknowledged the importance of positive selection in the origin of adaptive phenotypes. In a major review paper Kimura (1976, p. 445) wrote: 'It is true that adaptive change brought about by positive Darwinian selection is the most important aspect of biological evolution….In the preceeding [*sic*] sections, I have presented evidence suggesting that a majority of mutant substitutions that we observe at the molecular level are selectively neutral. This does not mean that adaptive changes do not occur at the level of information macromolecules. On the contrary, the marvelous function of molecular machineries on which life depends must be the products of positive Darwinian selection.'

In the same paper, Kimura (1976, p. 446) went on to remark, regarding the spread of advantageous mutants, that '[c]onsidering their great importance in evolution, it is perhaps surprising that well established cases are so scarce.' In the ensuing decades, a vast amount of molecular sequence data, including complete genome sequences of many organisms, has become available to test for the evidence of positive selection at the molecular level. However, the number of well-established cases has not increased greatly in comparison with those known in the mid-1970s (Hughes, 1999). It is true that a very large number of papers have been published in recent years purporting to show evidence of positive selection on the basis of various statistical methods. However, the vast majority of these cases cannot be considered well established. A variety of statistical tests for positive selection have been popular in recent years, including the so-called codon-based methods (Yang *et al.*, 2000), tests based on comparing polymorphism and divergence (Hudson *et al.*, 1987; McDonald and Kreitman, 1991), and various techniques to identify genomic regions of low heterozygosity or linkage disequilibrium (Sabeti *et al.*, 2006). A common feature of these tests is to search for a pattern that might be associated with positive selection under certain circumstances, but also may occur as a result of other factors in the complete absence of positive selection. Thus, they cannot be considered as true tests of the hypothesis of positive selection because they do not decide between

Department of Biological Sciences, University of South Carolina, Columbia, SC, USA
Correspondence: Dr AL Hughes, Department of Biological Sciences, University of South Carolina, Coker Life Sciences Bldg, 700 Sumter St, Columbia, SC 29208, USA.
E-mail: austin@biol.sc.edu
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positive selection and various alternatives (Nei, 2005; Jensen *et al.*, 2007; Hughes *et al.*, 2008; Hughes, 2007, 2008a; Nei *et al.*, 2010). Moreover, in almost all of the putative cases of positive selection identified by statistical analysis of sequence data alone, the biological basis of the supposed selection and even the phenotypic effects, if any, of the supposedly selected nucleotide substitutions have not been addressed (Hughes, 2007).

In fact, given the prominence accorded to the Neo-Darwinian mechanism in the biological literature, it may seem surprising that this mechanism has left so few unambiguous traces on the genomes of organisms (Nei *et al.*, 2010; Hernandez *et al.*, 2011). Rather, genomes show evidence that the predominant form of natural selection that occurs in populations is purifying selection; that is, natural selection acting to eliminate deleterious variants (Hughes *et al.*, 2003; Nei *et al.*, 2010). The predominance of purifying selection was predicted by Kimura and Ohta (1974), and the fact that their prediction has been proved to be correct is the cornerstone of many routine methods of modern bioinformatics, whereby evolutionary conservation of a sequence element (the consequence of purifying selection) is taken as evidence of that element's functional importance (Lesk, 2008).

Relatively few authors have suggested that adaptive phenotypes might arise in the absence of positive Darwinian selection. One such author was Nei (2007), when he proposed his 'new mutation theory of phenotypic evolution.' In the latter paper, as in earlier writings, Nei (1987, 2007) made the philosophical point that, even when positive selection operates, it is mutation that drives evolution. But Nei (2007) also suggested that there may be mechanisms by which new adaptive phenotypes can arise in the absence of positive selection. Here I consider in the form of a simple conceptual model one such mechanism, which I call the plasticity–relaxation–mutation (PRM) mechanism.

Although the genetic basis of phenotypic evolution remains an area of limited knowledge, I argue that substantial, though generally neglected, evidence for the generality of the PRM mechanism exists in the biological literature. I examine some predictions of this theory and summarize evidence relating to those predictions. The present hypothesis does not deny that the Neo-Darwinian mechanism operates in certain cases (Hughes, 1999). Rather, based on what we can learn from the known cases of positive selection, I conclude that the phenomenon of positive selection may be of relatively minor importance in phenotypic evolution. Instead, phenotypic plasticity and changes in the direction and nature of purifying selection, combined with the chance fixation of neutral or nearly neutral mutations, are proposed to be the major factors in the evolution of adaptive phenotypes. Before discussing the model, I discuss the definition of the term 'adaptation' and related terms in order to clarify the phenomenon that is being explained.

ADAPTATION AND RELATED CONCEPTS

Although biologists refer frequently to 'adaptation' and 'adaptive evolution,' these terms are rarely defined and have been defined by different authors in inconsistent ways, resulting in considerable confusion (Lewontin, 1957). Here I use the definition of Reeve and Sherman (1993, p. 9): 'An adaptation is a phenotypic variant that results in the highest fitness among a specified set of variants in a given environment.' Fitness in turn can be most directly measured as lifetime reproductive success, although in some cases reproductive success of close kin (inclusive fitness) should be included (Reeve and Sherman, 1993). This definition has the advantage that it makes it possible to express evolutionary theory in a way that is non-tautologous, by clearly differentiating between adaptation and the processes that give rise to adaptation (Reeve and Sherman, 1993).

As Reeve and Sherman (1993) point out, their definition has the important features of being both operational and non-historical. The definition is operational because it implies a means of testing the hypothesis of adaptiveness in the case of any given phenotypic variant, and thus of avoiding the all-too-common vice of 'adaptive storytelling' (Gould and Lewontin, 1979). Defined in this way, the hallmark of an adaptive phenotypic character is that, in a given environment, individuals lacking that specific character are at a disadvantage in terms of reproductive success, in comparison with those possessing that specific state of the trait, and evidence of such disadvantages can be straightforwardly obtained by ecological studies (Clutton-Brock, 1988).

The non-historical nature of Reeve and Sherman (1993) definition arises from the fact that it makes no assumption about the process that gave rise to the adaptative character, either ontogenetically (over the life of the organism) or phylogenetically (over the evolutionary history of the species). The same definition of adaptation can apply to a character that is directly genetically controlled, such as the presence of a given amino acid at a given site in a given protein. However, it can also apply to characters that arise through phenotypic plasticity, including those that result from learned behavior in animals. Consider the cases where animals have learned novel behaviors in response to novelties in the environment, which have then spread through the population via imitative learning. A well-known example involves the behavior of opening milk bottles by Great Tits in England, a behavior that is evidently adaptive in that it provides a new nutrient source (cream from the top of the bottle) unavailable to conspecifics lacking this behavior (Hawkins, 1950). Indeed, there are many known cases in which phenotypic plasticity gives rise to phenotypes, including both behavioral and morphological characters, which are adaptive in that they are conducive to reproductive success in a given environment (Hughes, 1985; Moczek, 1998; Mittelbach *et al.*, 1999; Nijhout, 2003; West-Eberhard, 2003).

An alternative definition of adaptation was given by Grant (1963, p. 93), who defined adaptation as the 'hereditary adjustment to the environment.' The concept here identified by Grant is an important one, but because I have already defined the term 'adaptation' in a different sense, I will use the term *evolved adaptation* for what Grant is referring to. An evolved adaptation is an adaptation that has, by an evolutionary process, become part of the genetically encoded heritage of a species. Thus, not every adaptive phenotypic character is an evolved adaptation, because some adaptive characters may result from phenotypic plasticity (including learning). Nonetheless, an important task for evolutionary biology is to uncover the mechanisms responsible for the origin of evolved adaptations. The PRM mechanism described below is proposed as one mechanism (along with the Neo-Darwinian mechanism) to account for the origin of evolved adaptations.

In a given case we may have evidence both (1) that a given character is adaptive, and (2) that the character is a genetically determined ('hard-wired') character of the species exhibiting it. In this case, we can be confident in stating that we are dealing with an evolved adaptation. But knowing that a character is an evolved adaptation is still not equivalent to knowing the evolutionary mechanism by which that adaptation arose. As emphasized by the philosopher Baublys (1975), 'Adaptedness and (the process of) adaptation are ….phenomena which it is the aim of evolutionary theory to explain.' In other words, even when we have evidence that a given character is an evolved adaptation, it remains a further step to test hypotheses regarding the mechanisms responsible for the evolutionary origin of that character.

THE PRM MODEL

Waddington (1953) observed a process of 'genetic assimilation,' whereby traits that were formerly environmentally determined somehow become genetically determined. Along with earlier ideas, such as those of Baldwin (1896), the importance of genetic assimilation in evolution remained controversial, largely because there was no clear understanding of the underlying genetic mechanism (Crispo, 2007). This concept was revived in a modern context in the groundbreaking work of West-Eberhard (1986, 2003, 2005), who pointed out that phenotypic plasticity might often precede the evolutionary origin of an evolved adaptation. Similar ideas have been developed by Pigliucci and Murren (2003), Pigliucci *et al.* (2006) and Pfennig *et al.* (2010). See Price *et al.* (2003); Lande (2009); Chevin *et al.* (2010) and Moczek *et al.* (2011) for additional perspectives on the evolutionary role of phenotypic plasticity.

Here I use the definition of phenotypic plasticity proposed by West-Eberhard (2003, p. 34): 'the ability of an organism to react to an environmental input with a change in form, state, movement, or rate of activity.' Thus, phenotypic plasticity can be defined as 'intra-individual variation…[including] adaptive and nonadaptive, active and passive, reversible and irreversible, and continuous and discontinuous responses' (West-Eberhard (2003, pp. 35, 36). The model presented here is based on processes discussed by West-Eberhard (1986) but purged of the Darwinian element, which I would argue is an unnecessary and even problematic feature of previous models.

Consider a situation where an organism expresses two different phenotypes when exposed to two different environments; this represents the classic scenario of phenotypic plasticity (Figure 1a). For the sake of simplicity, I consider the case of just two alternative phenotypes, recognizing that actual cases of phenotypic plasticity often involve a gradation of phenotypes corresponding to a gradient of environmental variation. In this simplified scenario, the organism expresses phenotype A′ in environment A, and phenotype B′ in environment B (Figure 1a). There is a genetic switch that determines which phenotype will be expressed as a result of input from the environment. Input from environment A leads the switch to initiate development along a developmental pathway (pathway *a*) leading to the expression of phenotype A′, whereas input from environment B leads the switch to initiate development along a developmental pathway (pathway *b*) leading to the development of phenotype B′ (Figure 1a).

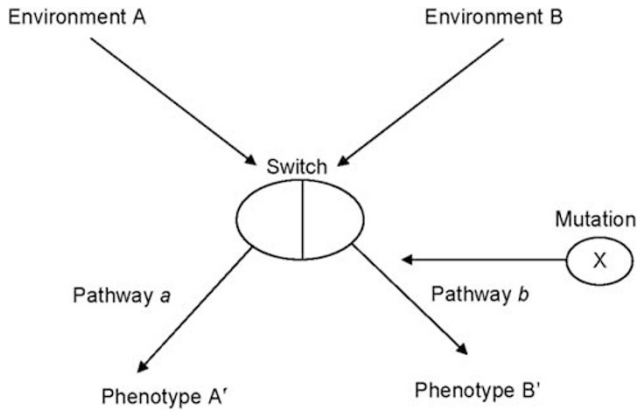

Figure 1 Schematic illustration of the PRM model. A genetic switch controls the expression of two alternate pathways (*a* and *b*) leading, respectively, to two alternate phenotypes (A′ and B′) in response to two different environments (A and B). When environment B is no longer encountered by the organism, there is no longer purifying selection against mutations (X) that eliminates pathway *b*.

I further assume that phenotype A′ is adapted to environment A in the sense that individuals expressing A′ in environment A do better (in terms of fitness) than do individuals expressing B′ in environment A. Conversely, individuals expressing B′ in environment A do better (in terms of fitness) than do individuals expressing A′ in environment B. Thus, by the definition of Reeve and Sherman (1993), phenotype A′ is an adaptation to environment A, in that it does better in A than does phenotype B′, and phenotype B′ is an adaptation to environment B in that it does better in B than does A′. However, there is no implication that either A′ or B′ is an 'evolved adaptation,' in the sense in which that term is defined in the previous section.

If there is an environmental change such that the organism now faces only environment A, a mutation that eliminates the possibility of pathway *b* (for example, X in Figure 1) will no longer be deleterious. Therefore, if such a mutation happens to occur, it may become fixed by genetic drift. Once this happens, phenotype A′ will become the 'genetically determined' phenotype of the species; in other words, genetic assimilation of a previously plastic phenotype will have occurred. Having previously been a phenotypically plastic response, phenotype A′ has now become an evolved adaptation (in the sense in which that term was defined in the previous section). The PRM mechanism thus involves ancestral phenotypic plasticity followed by specialization in one alternative environment and thus the permanent expression of one alternative phenotype. Once this specialization occurs, purifying selection on the molecular basis of other phenotypes is relaxed. Finally, mutations that permanently eliminate the pathways leading to alternative phenotypes can be fixed by genetic drift.

In West-Eberhard's (1986) model, following Waddington (1953), it is assumed that positive selection fixes the mutation that eliminates pathway *b*. But this assumption is unnecessary, because mutation and drift are likely to have the effect of eliminating pathway *b*, once there is no longer purifying selection on the components of pathway *b*. In fact, the hypothesis of positive selection in this case is problematic because, once the organism is no longer exposed to environment B, it is hard to see how positive selection favoring the loss of expression of phenotype B′ would operate. It might be argued that selection could favor the loss of pathway *b* if there is a cost involved in procuring the energy or materials needed to maintain this pathway, but such a cost is likely to be small. The persistence of numerous apparently inefficient mechanisms in gene expression and other aspects of cell biology (Lynch, 2007) suggests caution in applying cost-based evolutionary arguments to molecular mechanisms. In any event, the relaxation of purifying selection in the presence of environmental change is a more parsimonious hypothesis, of which there are numerous well-documented examples (Liman and Innan, 2003; Wang *et al.*, 2004; Collins and Bell, 2006; McBride, 2007).

Epigenetic mechanisms, such as DNA methylation, are known to have roles in phenotypic plasticity (Youngson *et al.*, 2010), and these mechanisms may be involved in the inactivation of the inappropriate phenotype as postulated in the PRM model. Indeed, epigenetic silencing of the genes involved in the disfavored pathway might be important in accelerating evolution by the PRM model because it quickly shelters genes from purifying selection and therefore allows mutations eliminating the unused pathway to drift to fixation. Epigenetic silencing of the genes involved in the disfavored pathway might even be passed to offspring by transgenerational epigenetic inheritance in the germline (Chong and Whitelaw, 2004; Richards, 2006), causing the developmental silencing of the pathway to be passed from parent to offspring even prior to the occurrence of any

silencing mutation. However, it is important to note that neither germline silencing nor the involvement of DNA methylation in general is a necessary feature of the PRM model.

It has been debated whether developmental plasticity is itself an adaptation, because plasticity might be advantageous under certain environmental circumstances (Via *et al.*, 1995; Schlichting and Smith, 2002). Although I do not deny that phenotypic plasticity may be an evolved adaptation in certain cases, evolution by the PRM model can occur as long as phenotypic plasticity is present, whatever its origin. Indeed, I would suggest that a certain degree of phenotypic plasticity is a characteristic of all living things, being a consequence of the following facts: (1) the development of any phenotype in even the simplest of organisms requires environmental input, and changes in the environmental input frequently alter the phenotype; and (2) developmental programs are modular in organization and initiated by molecular switch mechanisms (Carroll, 2008; Erwin and Davidson, 2009; Gilbert and Epel, 2009).

The biological literature provides abundant evidence of phenotypic plasticity in the apparent absence of evolved adaptation, including the following:

(1) Phenotypic plasticity has been observed in response to circumstances not corresponding to any that are regularly encountered in nature. For example, phenotypically plastic responses have been reported to occur in response to manipulations such as extreme heat shock (Lindquist, 1986), manner of food preparation (Beharka *et al.*, 1998), captive rearing (McKechnie *et al.*, 2006) and *in vitro* culture of cells (Iacovitti *et al.*, 1987; Peyton *et al.*, 2008). Another example is provided by the 'two-legged goat effect' (West-Eberhard, 2003), named for a goat that lacked forelimbs owing to a developmental anomaly but developed extensive muscular and skeletal adaptations for bipedalism (Slijper, 1942a, b). A similar example involved a chacma baboon (*Papio anubis*) whose forelimbs were paralyzed by polio (West-Eberhard, 2003). The two-legged goat effect illustrates the capacity of developmental programs to yield an adaptive phenotype even in an unnatural context (West-Eberhard, 2003, 2005).

(2) Tumor cells show evidence of phenotypic plasticity that is adaptive from the point of view of the tumor, in terms of processes such as tumor growth, survival and metastasis (Yacoby, 2005; Hudson *et al.*, 2008). The molecular basis of tumor plasticity remains poorly understood, although presumably it makes use of mechanisms that have a role in normal development (Feinberg, 2007). Nonetheless, the involvement of phenotypic plasticity in novel processes such as metastasis is evidence that plasticity can yield adaptive phenotypes with no previous evolutionary basis.

(3) Genome-wide studies of gene expression generally show that environmental changes affect the expression of large numbers of genes (for example, Causton *et al.*, 2001; Chapman *et al.*, 2011). Thus, a great many genes show phenotypic plasticity in expression (van Kleunen and Fischer, 2005). It is hard to imagine that phenotypically plastic expression represents an evolved adaptation in the case of every such gene (although any such case where the expression difference confers an advantage represents a potential future target for the PRM mechanism).

(4) Phenotypically plastic responses have been reported in nature in response to novel environmental situations different from any encountered in the species' evolutionary history. Examples include behavioral innovations in response to human-altered environments, such as the opening of milk bottles by the Great Tits discussed above (Hawkins, 1950) and plasticity of life-history traits in species invading novel environments (Yeh and Price, 2004).

The two-legged goat effect and the adaptive phenotypic plasticity of tumor cells (along with other examples, West-Eberhard, 2003) imply that, at least in some instances, phenotypic plasticity can give rise to phenotypes that are adaptive under the environmental circumstances triggering them, even in the absence of an evolved adaptation. However, as pointed out by an anonymous reviewer, the PRM mechanism might still work even if the phenotypes induced by given environments are completely random with respect to their fitness effects. In the simple model of Figure 1, we can imagine three possible fitness relations for phenotype A′: A′ is fitter than B′ in environment A; B′ is fitter than A′ in A; and A′ and B′ are equally fit in A. Assuming that these three states occur with equal probability, 33% of the time the fitness relations are such that the PRM mechanism can operate, leading to genetic assimilation of phenotype A′ if the species becomes exposed to environment A only. Such a random process may seem ineffective in giving rise to phenotypes of large adaptive effect; however, as in the standard Neo-Darwinian process, small effects can have a cumulative impact on over a long evolutionary time. Moreover, although 33% may seem a small probability, it is many orders of magnitude greater than the probability of occurrence of an advantageous mutation as required by the Neo-Darwinian mechanism.

PREDICTIONS

Given the opportunistic nature of the evolutionary process, it may be conceded that the PRM model is applicable in some cases. What is more difficult to assess given our present level of knowledge is how general this model is. Here I discuss several predictions that are likely to be supported if the PRM is of wide applicability.

1. *Many (perhaps most) evolved adaptations originate as exaptations.* Biologists have long used the term 'pre-adaptation' to refer to a trait that turns out fortuitously to be adaptive in an environment that the organism encounters subsequent to the evolution of the trait. However, as Gould and Vrba (1982) noted, many biologists expressed discomfort with the term 'pre-adaptation' because of its apparent teleological connotations. Gould and Vrba (1982) thus proposed the term 'exaptation' for a pre-existing trait that is 'co-opted' for a new function. The use of the pre-existing trait in a new context can only occur when the phenotype is plastic. Thus, the frequent occurrence of exaptation preceding adaptation is consistent with the current model. Here I briefly discuss several lines of evidence regarding the role of exaptation in evolution.

Anthropogenic alteration of the environment provides a large-scale natural experiment in evolution (Myers and Knoll, 2001) that provides evidence for the prevalence of exaptation. Verified cases of positive Darwinian selection in response to human-induced environmental changes have been relatively few, mostly involving drugs, pesticides and other toxins (Mita *et al.*, 2009; Rivero *et al.*, 2010). Rather, the main biotic result of human-induced environmental alteration has been 'a few winners replacing many losers,' in the memorable phrase of McKinney and Lockwood, 1999. Numerous studies have sought to identify the characteristics of the 'winner' species, and the results have suggested that they tend to be 'broadly adapted ecological generalists' (McKinney and Lockwood, 1999). It is important to note that these are species-typical characteristics present in the species prior to human alteration of the environment, and thus are exaptations. Interestingly, there is evidence that similar characteristics enabled species to survive past mass extinction events that were not human-induced (Erwin, 1998). Another aspect of the human alteration of the environment that addresses the issue of exaptation is that of invasive species, and a large literature has attempted to identify common traits of successful invaders (Williamson and Fitter, 1996; Sax and Brown, 2000; Kolar and Lodge, 2001; Blackburn *et al.*, 2009). In general, the

traits of successful invaders are already possessed prior to introduction to a new environment and thus are exaptations.

Moreover, several recent studies have provided evidence that successful radiations in the evolutionary past have been due to traits present in the ancestors before the radiation. For example, a phylogenetic analysis of toads (Anura: Bufonidae) identified a suite of characters correlated with range expansion that were inferred to have been present in the ancestors of the family prior to its global expansion (Van Bocxlaer *et al.*, 2010). Similarly, rapid expansion of the avian family Zosteropidae through the Old World tropics was attributed to traits present in the family's common ancestor (Moyle *et al.*, 2009).

2. *An important aspect of organismal adaptedness to a given environment consists of the inability to inhabit other environments rather than some kind of optimal 'fit' to the environment currently inhabited.* On the present hypothesis, adaptedness to a particular habitat or ecological niche is to a substantial degree a negative phenomenon, reflecting the loss of broader adaptedness as a result of mutation and genetic drift. Naturalists have long been aware of the negative side of adaptedness. For example, Grant (1963, p. 93) writes: 'Every kind of organism occurs in a particular habitat; removed from its normal habitat it is like a fish out of water.' The drift to fixation of mutations eliminating traits that are not useful in a given niche is well supported by evidence from molecular evolutionary studies; for example, the loss of eyes in cave-inhabiting fishes (Jeffrey, 2001). The simplification of the genome of the sessile organism *Ciona intestinalis* through loss of numerous ancestral genes may represent a similar phenomenon (Hughes and Friedman, 2005). The morphological simplification of morphology in species specialized for a sessile life (such as clams and barnacles) or as endoparasites (such as tapeworms) is well known, and it is a plausible hypothesis that such changes have resulted from mutations leading to the loss of ancestral genes and developmental pathways. The endosymbiotic parasite *Mycobacterium leprae* provides evidence of such a process at the genomic level, with massive decay of genes involved in pathways provided by the host (Vissa and Brennan, 2001).

3. *Cases of phenotypic plasticity preceding the fixation of evolved adaptations are predicted to be widespread.* A number of studies have described groups of closely related taxa, some of which show phenotypic plasticity with respect to a given trait, whereas others show apparent fixation of just one of the alternative phenotypes. For example, populations of tiger snakes (*Notechis scutatus*) that have colonized different islands at times ranging from 30 to 9000 years ago show differences in head size that are correlated with prey size. In recently colonized islands, large head size (conferring the ability to ingest large prey) is achieved by phenotypic plasticity, whereas in older populations the plasticity is lost and large head size has been genetically assimilated (Aubret and Shine, 2009). Similarly, in spadefoot toads (*Spea* sp.) there is evidence that phenotypic plasticity with respect to larval diet and gut morphology preceded the evolution of species whose larvae showed a greater tendency toward carnivory (Ledon-Rettig *et al.*, 2008). Similarly, there is evidence that phenotypic plasticity preceded evolution of morphologically distinct ecotypes in threespine sticklebacks *Gasterosteus aculeatus* (Wund *et al.*, 2008). Moczek *et al.* (2011) summarize numerous cases in which an evolutionary innovation in a given lineage occurs as a facultatively expressed phenotype in a related lineage.

4. *Key genetic events underlying the evolution of phenotypic novelties will generally involve loss (for example, gene deletion, loss of expression in a given tissue, and so on) rather than gain.* A striking example of the role of gene loss in a major evolutionary transition is provided by the loss of the actinodin 1 and 2 genes in tetrapods (Zhang *et al.*, 2010). The products of these genes, which are present in both cartilaginous and bony fishes, are essential for fin development in bony fishes, and their loss in tetrapods appears to have facilitated the morphological transition from fins to limbs (Zhang *et al.*, 2010). It is possible that expression of these genes was phenotypically plastic in the ancestor of tetrapods, allowing for mutations leading to their elimination in a population where they were no longer expressed. Recent evidence that numerous human-specific traits are the result of the loss of regulatory sequences conserved in other mammals (McClean *et al.*, 2011) is likewise consistent with this prediction.

DISCUSSION

The PRM model is non-Darwinian in that it does not rely on the mechanism of positive selection. It is worth emphasizing that this model is also non-Lamarckian, because it does not postulate environmentally directed alteration of the genome; neither does it rely on unrealistic concepts, such as orthogenesis or macromutation. Rather, this mechanism relies only on the processes of purifying selection, mutation and genetic drift, for which genomes show abundant evidence, but it does not depend on the mechanism of positive selection, for which conclusive evidence is rare. I do not question the operation of the Neo-Darwinism mechanism in certain cases (Hughes, 1999), nor that the Neo-Darwinian mechanism is in principle sufficient for the origin of an evolved adaptation. But if the PRM mechanism has operated even occasionally, we must conclude that the Neo-Darwinian mechanism is not necessary for the origin of an evolved adaptation.

Cases where an evolutionary innovation in a given lineage occurs as a facultatively expressed phenotype in a related lineage (Moczek *et al.*, 2011) provide the best opportunity for a critical test of the operation of the PRM mechanism. In these cases, the PRM mechanism predicts a relaxation of purifying selection on pathways giving rise to the phenotype that has been lost in the former lineage. The availability of closely related species should make it possible to reconstruct the ancestral state of the relevant pathways by standard phylogenetic methods. The photosynthetic mechanisms of flowering plants would seem to provide an especially fruitful model system for this research. The alternative CAM and C_4 pathways have evolved independently in multiple lineages where the C_3 pathway was ancestral, and there are known species in which the occurrence of CAM photosynthesis is phenotypically plastic (West-Eberhard *et al.*, 2011). Therefore, there is a potential for numerous phylogenetically independent comparisons of CAM or C_4 species with related C_3 species.

The PRM mechanism provides unification to the biological sciences by uniting observations at the genomic level (where purifying selection and genetic drift predominate) with those at the phenotypic level (where adaptive characters are well known). As mentioned above, some known examples are suggestive of the action of the PRM mechanism, but it is not yet known how widespread this mechanism is. However, I would predict that the PRM mechanism is likely to be a major mechanism for the origin of evolved adaptations, and perhaps more common than the Neo-Darwinian mechanism.

Widespread occurrence of the PRM mechanism would easily explain recently reported cases of apparent phenotypic evolution over ecological time (Reznick and Ghalambor, 2001; Hairston *et al.*, 2005; Carroll *et al.*, 2007; Schoener, 2011). In most such cases, there has been no genetic evidence demonstrating the operation of the classic Neo-Darwinian mechanism of allelic replacement. In some cases, the time frame seems rather short for a Darwinian process to have occurred, and in other cases, the effective population sizes of the species in question are small, suggesting that there is unlikely to have

been extensive genetic variation in the population prior to selection (Willi *et al.*, 2006). However, none of these factors are problematic if these cases of apparent rapid evolution in fact represent cases of phenotypic plasticity, perhaps in some cases rendered heritable through germline DNA methylation. Thus, rather than the paradoxical observation of Darwinian evolution over ecological time, we may be merely seeing incipient evolution by the PRM mechanism, which is expected to operate over ecological time. The same process might also be involved in rapid responses to artificial selection, for instance in accelerated domestication (Belyaev, 1979).

The PRM mechanism provides a simple explanation of such comparatively recent adaptive radiations as that of the cichlids of the East African Great Lakes. The oldest of these lakes, Lake Victoria, is no more than 200 000 years old, and others are still more recent (Sturmbauer, 1998). The diversity of species in these lakes is problematic for Neo-Darwinism, but is easily explained by the PRM mechanism if prior to the divergence of ecotypes the ancestral species showed a phenotypic plasticity similar to that described in sticklebacks (Wund *et al.*, 2008). Perhaps ironically, the PRM mechanism can likewise account readily for the radiation of Darwin's finches in the Galápagos Islands. The natural history of Darwin's finches provides many examples where it is plausible that phenotypic plasticity preceded morphological change; a striking example involves the sharper bill shape of a population of the ground finch *Geospiza diffilis* that feeds on the blood of boobies (Schluter and Grant, 1984).

In addition to its explanatory power regarding specific cases of adaptive evolution, the theoretical perspective developed here may aid concept clarification in biology. I have mentioned previously the confusion in the biological literature regarding the term 'adaptation' and the desirability of an operational definition. As mentioned, at the phenotypic level, such an operational definition implies that the adaptiveness of a given trait principle is testable by ecological studies. It is also worth pointing out that, at the molecular level, the hallmark of an evolved adaptation is purifying selection, because purifying selection is evidence that mutations altering the trait are disfavored. This is true whether the trait has arisen by the Neo-Darwinian mechanism, the PRM mechanism or any other process. As the genomic 'signatures' of purifying selection are relatively unambiguous (Hughes *et al.*, 2003; Hughes, 2005, 2008b), the use of purifying selection as a criterion for the presence of evolved adaptation provides an operational definition for a term that has previously been difficult for biologists to define.

When Darwin published his *Origin of Species*, both the hypothesis of organic evolution and his proposed mechanism (natural selection) were new and controversial ideas. Now no serious scientist questions the fact that organic evolution has occurred; however, as our knowledge of evolution has increased, the generality of the Neo-Darwinian mechanism has become increasingly debatable (Nei, 2005). The hypothesis proposed here has the advantage of explaining the available data regarding adaptive evolution on the levels of genomics, ecology and paleontology, without invoking any mechanisms other than the commonly observed phenomena of phenotypic plasticity, purifying selection, mutation and genetic drift. Although it may represent a new perspective to biologists schooled in Neo-Darwinism, this view of life in its own way is not without 'a certain grandeur.'

CONFLICT OF INTEREST

The author declares no conflict of interest.

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