Cryptic genetic variation: evolution's hidden substrate

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Abstract | Cryptic genetic variation (CGV) is invisible under normal conditions, but it can fuel evolution when circumstances change. In theory, CGV can represent a massive cache of adaptive potential or a pool of deleterious alleles that are in need of constant suppression. CGV emerges from both neutral and selective processes, and it may inform about how human populations respond to change. CGV facilitates adaptation in experimental settings, but does it have an important role in the real world? Here, we review the empirical support for widespread CGV in natural populations, including its potential role in emerging human diseases and the growing evidence of its contribution to evolution.

Standing genetic variation
Genetic variation that is present in a population, as opposed to new mutations.

Additive genetic variance
(V_A). The transmissible or heritable component of the phenotypic variation of a population. This is the variation due to the additive effects of segregating alleles.

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Cryptic genetic variation (CGV) is genetic variation that normally has little or no effect on phenotypic variation but that under atypical conditions, rare in the history of a population, generates heritable phenotypic variation. Although CGV is often perceived as mechanistically special and mysterious, it is simply a subclass of variation with conditional effects, which has two well-studied forms: gene-by-gene (G×G) interactions, including dominance and epistasis, in which the effect of an allele is conditional on genetic background; and gene-by-environment (G×E) interactions, in which the effect of an allele is conditional on the environment (FIG. 1). The distinguishing feature of CGV is that the conditions that induce allelic effects are rare or absent in the history of the population, and this rarity limits the opportunities for selection to act on the variation and allows it to accumulate. CGV then provides a pool of standing genetic variation poised to facilitate adaptation when the rare condition becomes common. Variation that is hidden from selection may alternatively be maladaptive in the new condition, which underlies the hypothesis that modern environments increase the genetic contribution to human disease risk[1].

The definition of CGV encompasses both molecular and quantitative genetic perspectives. From the molecular genetics view, cryptic genetic variants are polymorphic loci that have no effect on phenotype until they are perturbed by unusual conditions[2]. From the quantitative genetics view, cryptic genetic variance is an increase in additive genetic variance (V_A; that is, the heritable phenotypic variation) that arises when a population is exposed to unusual conditions[3]. This distinction between variants (that is, discrete loci with segregating alleles) and variance (that is, V_A) parallels the difference between compositional and statistical epistasis[4]; variants deal with the genotype–phenotype map, whereas variance concerns heritability in populations. Both forms are relevant to CGV.

Early investigations into CGV and their implications are thoroughly discussed elsewhere[2]; here, we briefly summarize the historically provocative role of CGV in evolutionary theory and the mechanisms by which CGV may accumulate. Most of the research we review derives from work in sexual, outcrossing species, for which CGV is most likely to be important in adaptation[5]. We focus our Review on the seemingly vast extent of CGV in nature and the role of such variation in adaptation and disease, which is currently less clear.

The CGV legacy

The existence of CGV is a long-standing subject of study in evolutionary genetics that is motivated by a need to explain the ability of populations to adapt. Why would a population harbour variation that is adaptive in an environment it has never encountered? CGV provided a solution. In 1941, T. Dobzhansky[6] listed J. B. S. Haldane and the Russian geneticist N. J. Shapiro, along with himself, as advocates of "a store of concealed genetic variability" containing mutations that were invisible when they arose but that may turn beneficial under new circumstances.

Modern enthusiasm for CGV builds on the iconic work of C. H. Waddington, who provided a clear evolutionary scenario to account for abundant CGV. Waddington noted that, as an empirical matter of fact, wild-type phenotypes develop robustly with

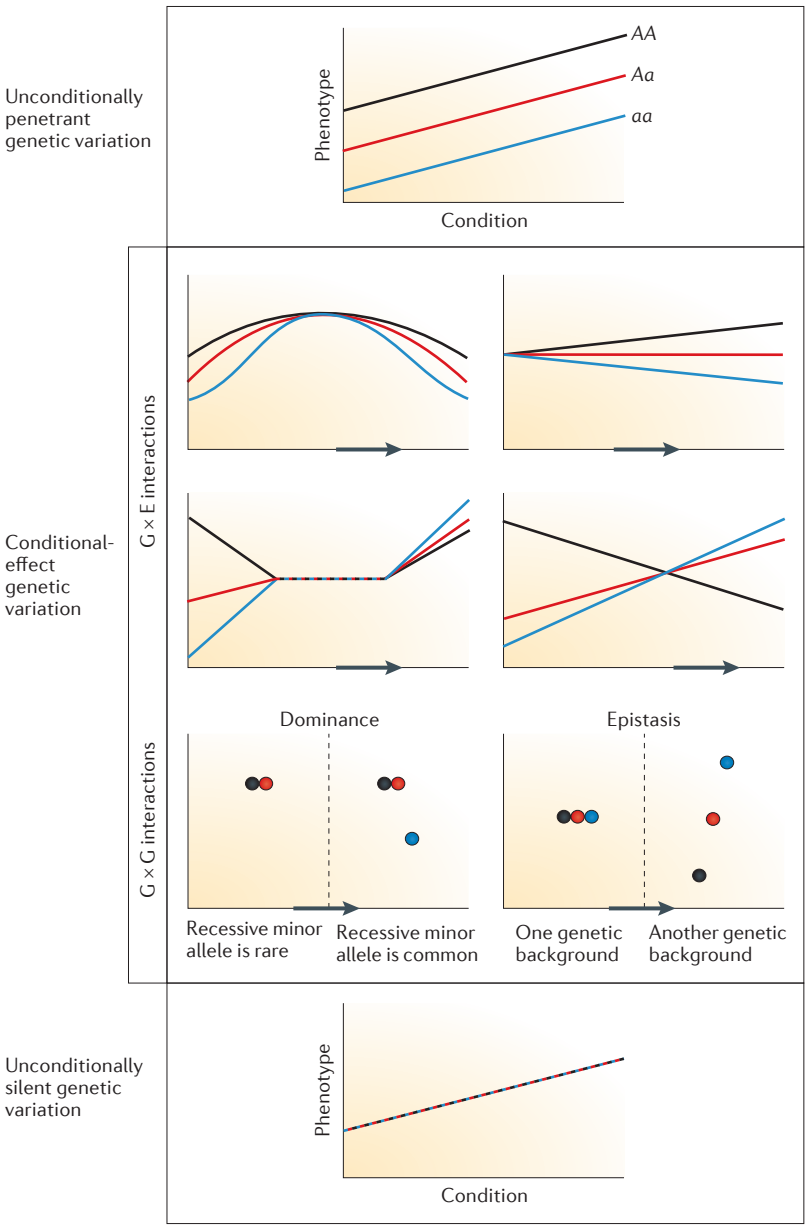

Figure 1 | **Cryptic genetic variation is conditional-effect genetic variation.** A few of the infinite possibilities for conditional-effect genetic variation are shown. Each plot shows phenotype (*y* axis) as a function of condition (either environment or genetic background; *x* axis) for three genotypes (*AA* (homozygote); *Aa* (heterozygote); *aa* (homozygote)) at a locus. The top panel shows unconditionally penetrant genetic variation, which affects phenotype independently of condition. The bottom panel shows unconditionally silent variation, which has no effect under any circumstances, and the three lines are therefore superimposed. Between these extremes are variants with effects that are dependent on circumstances. In each of the six scenarios shown (middle panel), as conditions change (represented by the arrows along the *x* axes) cryptic genetic variation is revealed. In some cases, the genetic variants are completely cryptic in the initial condition, whereas in others their effect sizes change across conditions. In the top left panel in this cluster, the population has no heritable variation at the start of the arrow, but a population in the condition at the end of the arrow shows heritable variation; in the panel to its right, a population at the start of the arrow harbours some heritable phenotypic variation, but the variance increases when the population is subjected to different conditions at the end of the arrow. Variation can be exposed either by gene-by-environment (G × E) interactions or by gene-by-gene (G × G) interactions (that is, changes in genetic background, including dominance and epistasis).

little variation[7]. The insensitivity of the wild type, he argued, is the result of evolved buffering mechanisms. If departures from the present-day optimum are disadvantageous, stabilizing selection will favour the evolution of mechanisms that dampen the effects of such perturbations, which yields a nearly invariant or canalized phenotype. Crucially, Waddington showed that when organisms are pushed well outside their ordinary conditions and their dampening mechanisms are overwhelmed, they show heritable phenotypic variation that had been invisible but that is present all along. Waddington's idea that stabilizing selection generates CGV is well supported by population genetic models[3].

Waddington argued that given canalization, an alternative path to adaptive evolution known as genetic assimilation could occur. Using heat shock to induce changes in *Drosophila melanogaster* wing veination[8], and separately using ether to induce homeotic transformations of body parts[9], he observed variation across heterogeneous lines and artificially selected the most extreme phenotypes. Eventually, the phenotypes were 'captured', and the selected lines no longer required the stimulus to express those phenotypes. Decades later, these early experiments inspired a modern inquiry into CGV. The first gene shown to harbour CGV was *Ultrabithorax*[10], and the first cryptic variants at the nucleotide level were identified in the *Epidermal growth factor receptor* gene[11], both in *D. melanogaster*. In the same species, disruption of the heat shock chaperone protein Hsp90 (also known as Hsp83) was shown to release CGV[12], which started a major research programme into the role of this protein as a buffering mechanism (BOX 1; see below). These experiments provided a proof of principle for the adaptive potential of CGV (FIG. 2).

How does CGV accumulate?

Waddington's model of canalization invokes buffering mechanisms that conceal CGV. There are two kinds of evolved buffering mechanisms that are each supported by empirical evidence[13]. First, under normal conditions, a population may evolve systems that suppress any and all departures from the wild type. Hsp90, which suppresses the effects of misfolded proteins, remains the prominent example of such a generic buffering mechanism (BOX 1). Generic buffering systems are called capacitors[12], as they have the potential to suppress, and thereby store, an enormous charge of variation that can be released when perturbed. A second type of buffering can arise if stabilizing selection favours mechanisms that suppress perturbations to individual phenotypes. Such targeted suppression could involve phenotype-specific gene networks through the evolution of duplicate genes, redundant pathways or shadow enhancers[14]. For example, a *cis*-regulatory region at the *D. melanogaster shavenbaby* locus is mostly superfluous under ideal conditions but is necessary to preserve wild-type expression under thermal stress[15]. Generic and specific buffering mechanisms thus have different implications for the nature of the stored variation and the perturbations that could expose it.

Box 1 | **The Hsp90 story**

Rutherford and Lindquist's discovery that reducing activity of the heat shock chaperone protein Hsp90 releases cryptic genetic variation (CGV) in *Drosophila melanogaster*[12] motivated a renewed experimental effort in the investigation of genetic assimilation and, in particular, the use of Hsp90 as a buffering mechanism. Reduced Hsp90 activity has also been shown to release CGV for phenotypes in *Arabidopsis thaliana*[97] (FIG. 3), cave fish[91] and yeast[87], and to increase the severity of developmental mutations in zebrafish[98]. Hsp90 provides a straightforward mechanism for buffering the effects of CGV. As a chaperone, Hsp90 assists in folding other proteins and in refolding misfolded proteins. Mutations in coding sequence can lead to folding error, and reduction in chaperone activity should therefore increase the expression and penetrance of protein-coding mutations.

However, two main criticisms have been levied at Hsp90 as a model for releasing CGV and promoting genetic assimilation. One criticism is that reduction of Hsp90 activity affects biogenesis of PIWI-interacting RNA, which in turn permits transposable element activity in the germ line and can lead to *de novo*, heritable mutations[99]. If new mutations account for the variation in Hsp90 knockdowns, then the best-studied example of CGV no longer stands. The second criticism is that Hsp90 may be exceptional and is therefore not a general model for buffering. Hsp90 is an abundant protein and interacts with many molecules in the cell[100]. Are there other genes that can demonstrate similar buffering of standing genetic variation? And how relevant is synthetic depletion of Hsp90 to the adaptive dynamics of natural populations?

Several studies have found that naturally occurring polymorphisms in Hsp90 can affect fitness and morphology[101,102], and that natural environmental perturbations, such as the low conductivity in aquatic cave habitats, can reduce Hsp90 function[91]. Other cryptic genetic variants revealed by chemical inhibition of Hsp90 have been confirmed as pre-existing[87], and evidence also suggests that the reduction in Hsp90 that is required to affect transposable element activity is greater than that necessary to reveal CGV[103]. These results show that Hsp90 is a legitimate proof of principle for CGV. Currently, Hsp90 remains the most prominent example of a mechanism that reveals CGV, but recent experiments in *D. melanogaster* suggest that many genes can function to hide CGV at other loci[96,104]. Future work in this promising area will surely show whether Hsp90 is unique or whether it is simply an early herald of an important evolutionary mechanism.

However, buffering mechanisms are not required for CGV to accumulate. For example, conditional-effect alleles may arise routinely as new mutations and, in the absence of phenotypic effects, their frequencies are only subject to genetic drift. The pool of CGV in a population is then determined by the product of effective population size, the mutation rate and the proportion of mutations that have conditional effects. The latter is determined by both the biochemical properties of the mutant alleles and the cellular networks in which they reside. For example, biological macromolecules are sensitive to temperature, pH and ion concentrations in a nonlinear manner. Independently, the architecture of pathways and networks can generate automatic conditional neutrality for a large proportion of mutations in the absence of any evolved buffering mechanism[16,17].

In short, under this neutralist scenario, populations may harbour CGV merely because alleles have never been subjected to selection. An extreme example is a rare recessive allele that is deleterious in the homozygous state in its present environment and that is maintained only by mutation–selection-drift balance. Such alleles achieve higher frequencies than additive-effect alleles because their recessive nature makes them cryptic and, when conditions changes, they can provide the 'raw material' for adaptation[18].

Stabilizing selection
Natural selection that favours an intermediate phenotype and that disfavours phenotypes which depart from it in any direction.

Canalized
Pertaining to canalization, which is the evolved resistance to perturbations, such that an invariant phenotype is produced across a range of genotypes and environments.

Genetic assimilation
The process by which selection converts phenotypes that are revealed by environmental stimuli into phenotypes that are reliably produced in the absence of those stimuli. It relies on genetic variation revealed by those stimuli.

This example of a recessive allele recalls the classic Fisher–Wright debate over the evolution of dominance: why are new mutations usually recessive? Fisher favoured evolved suppressors, and Wright favoured a biochemical explanation. In this debate, which precisely echoes that between evolved and neutral CGV, Wright's position was vindicated[19,20]. However, in the context of CGV, the two positions are not mutually exclusive and both are likely to contain some truth.

Moreover, it is important to note that the role of CGV in adaptation and disease depends only on its actual realized properties and abundance but not on the mechanisms that create it. CGV is a class of variation rather than a process. In other words, we can set aside debate over robustness, canalization, buffering and capacitance — phenomena that may facilitate the accumulation of CGV but that have contested relationships with such variation (BOX 2). We do not need to know why CGV exists to ask whether it is important[3,21].

What does CGV look like?

If populations harbour genetic variation that is normally invisible and that is only evident when the population experiences novel conditions, then direct experimental manipulation of conditions should reveal it. The foundational work in *D. melanogaster* showed this clearly for morphological traits; here, we review more recent experiments in other systems that uncover and characterize CGV.

The nematode vulva: a model for CGV. The vulva is the egg-laying and copulatory organ in *Caenorhabditis elegans* hermaphrodites and is formed from ventral epidermal precursor cells that undergo highly canalized cell fate specification. Six cells are competent to adopt the vulva fate but, under normal conditions, only three do so in response to RAS pathway signalling and morphogen secretion from the gonadal anchor cell. Using mutations and laser ablation of the anchor cell to perturb vulva development, one study[22] observed CGV in cell fate specification across wild *C. elegans* isolates and found that both the number of cells achieving the vulva fate and the timing of their induction differed markedly across strains.

Work in this system elucidates aspects of CGV that may be generalizable. Although the wild *C. elegans* isolates have morphologically invariant vulval phenotypes under normal conditions, they show a twofold difference in RAS pathway activity[22]. This is a probable example of variation in an 'intermediate' phenotype, which is tolerated either because the differences are too small to disrupt the robust trait or because they are compensated for elsewhere; searches for variation in intermediate phenotypes may lead to new discovery of CGV[23]. Surveys for hypervariable traits across closely related taxa may also indicate sources of CGV. In the vulva, mechanisms that control cell fate specifications which have evolved most rapidly across species are also the most vulnerable to perturbation in *C. elegans*[24,25]. Cell ablations that were carried out on different members of the *Caenorhabditis* genus revealed different cell induction patterns, which indicates

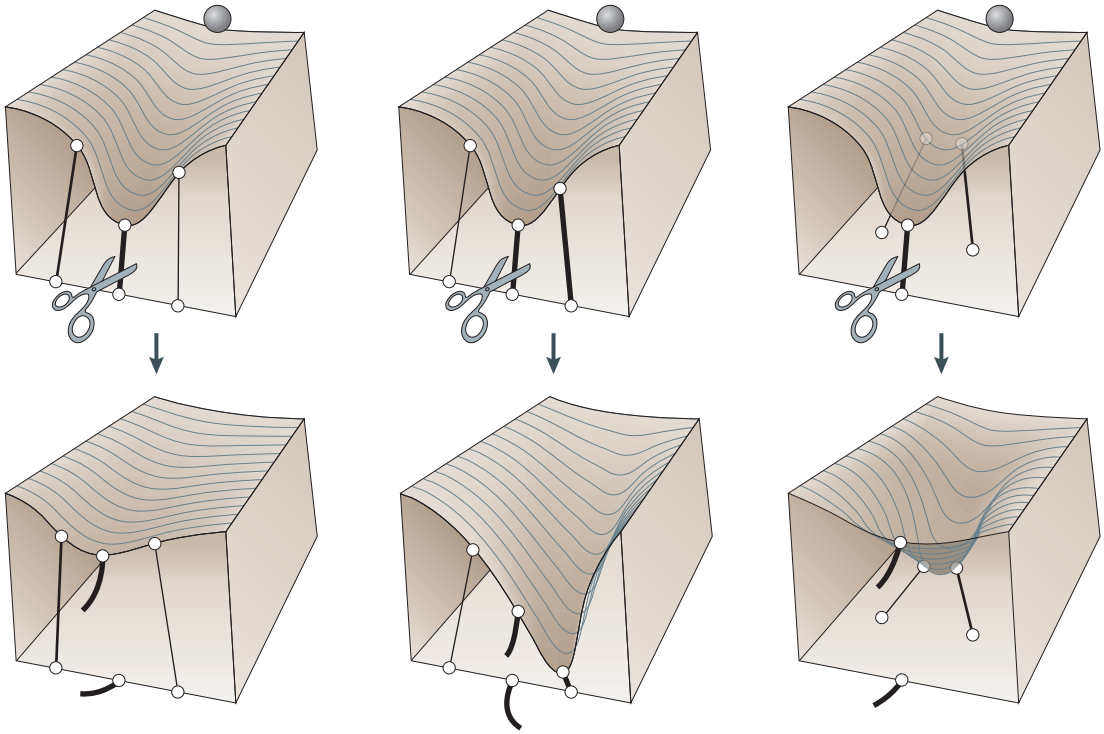

Figure 2 | **Waddington's epigenetic landscape, repurposed.** Waddington's original conception of canalization arose from his observation that as the embryo develops, tissues adopt discrete types such as the eye or the gut but never an intermediate[115]. His classic illustration depicts a ball atop a bifurcating landscape that is poised to roll down the path of the least resistance into valleys (also known as canals), the end points of which represent terminal differentiation. His literal depiction of genetic underpinnings shows guy-ropes pulling down, from the underside of the bifurcating landscape, the undulating topology of the valleys and fastening to anchors (shown as white circles) that represent genes[7]. Here, we repurpose Waddington's landscape to illustrate how cryptic genetic underpinnings can induce different phenotypic fates. These genetic underpinnings vary at the molecular level (represented by guy-ropes of different thickness and configurations) but produce a consistent phenotype. After disruption (depicted by breakage of the main rope, which represents a null mutation in a major gene), variation elsewhere produces deformities to the landscape.

Capacitors
Genes that conceal the phenotypic effects of mutations at other loci, allowing the population to build up a store of cryptic genetic variation available for evolutionary response when a capacitor is overcome by environmental challenge or mutation.

Mutation–selection-drift balance
An equilibrium that arises from the balance between the introduction of alleles by mutation and their elimination by genetic drift and natural selection.

Robustness
A state of reduced phenotypic variance, not necessarily evolved, which can be defined relative either to specific perturbations (such as standing genetic variation) or to perturbations in general (such as the full mutational spectrum).

divergence in the underlying mechanisms even though the pathways are conserved and the final vulva phenotype is morphologically invariant[26]. This is an example of developmental system drift, which is the interspecific analogue to intraspecific CGV and for which the nematode vulva provides an excellent model (BOX 3).

CGV has also been observed in the sex determination pathway of *C. elegans* which, unlike most of its relatives, shows a male-hermaphrodite mating system. Mutations at two known sex determination genes revealed hidden variation, and quantitative trait locus (QTL) mapping identified genomic regions both with and without known genes for sex determination[27]. The emergence of *C. elegans*, in addition to *D. melanogaster*, as a model for CGV studies suggests that CGV is a general feature of populations that can be easily accessed in genetically tractable organisms but that is probably also abundant in others.

Observations of increased variance. CGV can be inferred from changes in V_A across conditions. The estimation of V_A does not require sophisticated molecular tools and can provide evidence for the existence of CGV without attempting to identify causal loci. As V_A represents the transmissible component of phenotypic variation, an increase in V_A under perturbation indicates the presence of conditional, functional genetic variants. Whereas the previous examples demonstrate CGV by the transformation of invariant phenotypes into variant (and often aberrant) ones, the estimation of V_A also allows the possibility of phenotypic variation before perturbation. Several recent studies have shown that ecologically relevant changes to the environment can increase V_A in natural populations, including body size in sticklebacks[28], spermathecae number in dung flies[29], plasma antioxidant level in gulls[30] and traits that are associated with facultative carnivory in spadefoot toad relatives[31] (FIG. 3).

How much CGV do populations harbour?

The experiments described above involve targeted efforts to identify CGV. However, the broader question of the abundance of CGV can be addressed in a more general way by asking about the prevalence of its proximate genetic mechanisms[2,3]. In other words, are alleles with G × G and G × E interactions common? We should focus particularly on interactions that have been rarely tested in the history of a population.

Box 2 | **Cryptic genetic variation and robustness**

Robustness describes the relative insensitivity of a system to perturbation, and a robust genotype is one that shows little phenotypic variance. Studies of the genetics and evolution of robustness have historically used observations of cryptic genetic variation (CGV) as evidence that a system is robust. The logic is that the release of CGV shows that those strains or genotypes are phenotypically stable in the face of mutational perturbation because, until they were pushed beyond their tolerance, they hid genetic variation beneath a stable wild-type phenotype.

However, empirical observations of CGV yield little insight into the state of robustness[3,21]. Strictly speaking, CGV that is revealed by perturbation shows that the unperturbed system is robust to that specific collection of CGV. However, it bears no evidence for robustness against other genetic variation, including any new, untested mutations from across the spectrum that may occur in the future. Existing CGV samples the subset of mutations to which the wild type happens to be robust. Consequently, observations of CGV cannot provide evidence of general robustness.

Studies of robustness also include observations that phenotypic variance can be increased under stressful or extreme environments (reviewed in REF. 66). Nevertheless, some stressful conditions decrease phenotypic variance[67], analogous to the way that perturbations to the heat shock chaperone protein Hsp90 can both increase and decrease phenotypic variation across genetically distinct yeast lines[87] (K. Geiler-Samerotte, personal communication). A useful way of distinguishing between CGV (which is a class of genetic variation) and robustness (which is a systems-level property) is to recognize that CGV is simply conditionally neutral genetic variation. In theory, CGV could be revealed if conditions change and if silent mutations become visible, even under increased robustness. This scenario would arise if the new condition — even as it increased additive genetic variation from existing alleles — nevertheless sheltered the expression of other mutations, either in the future or in the present[21]. Specific relationships between CGV and the conditions that reveal it are not necessarily generalizable to other scenarios that affect phenotypic variance.

Similar to how CGV is not necessarily evidence for robustness, neither is it evidence for canalization, which is the evolved resistance to perturbation. Canalization can emerge either through positive selection on buffering mechanisms or simply under stabilizing selection in which the presence of gene-by-gene or gene-by-environment interactions permit accumulation of CGV[105]. The process of canalization will promote accumulation of CGV, but its presence does not indicate an evolved resistance to perturbation or a guaranteed resistance to other perturbations.

There are two broad classes of G × G interactions that have rarely been tested: higher-order epistasis, in which alleles at multiple loci may all be at intermediate frequencies, but particular genotypes may nevertheless be vanishingly rare; and modifiers of rare mutations, which are well studied in the context of human Mendelian diseases.

Higher-order epistasis. Recent empirical data suggest that higher-order epistasis is exceptionally abundant, even if its effects are rarely exposed. A key type of evidence comes from near-isogenic lines (NILs; also known as congenics) and chromosome substitution strains (CSSs; also known as consomics), which are inbred lines carrying a fragment of one wild-type genome that has been introgressed into a different wild-type genetic background. Studies in mice have found that these isolated genomic regions have large phenotypic effects in these heterologous backgrounds, and such effects can vastly exceed their additive effects averaged across backgrounds[32,33]. For example, one study[32] found that for 20 of 90 traits of mouse blood, bone and metabolism, introgressing a chromosome from one strain into another resulted in an effect that exceeded the phenotypic difference between the two strains. For two of these traits, seven different chromosome substitutions each had such large effects.

The genomic regions in mouse CSSs often contain multiple separable genetic effects that are tightly linked[32], which suggests that linkage disequilibrium can store CGV that can be released by recombination[34,35]. Similar findings have emerged from analyses of NILs in *Arabidopsis thaliana* and *C. elegans*[36–38]. The same conclusions can be drawn from other experimental designs, including the comparison of additive genetic effects between inbred lines and outbred populations of *D. melanogaster*[39], and the genetic complexity of mutational suppression in a cross of yeast strains[40]. In general, individuals with rare, untested genotypic combinations often show new phenotypes — a principle that has also been demonstrated by transgressive segregation in genetic crosses[41].

In these examples, the epistatic effects are probably a by-product of stabilizing selection, as the phenotypic variance is low across progenitors. Stabilizing selection within isolated lineages can produce eventual incompatibilities between hybrids, even as the polygenic trait in question maintains a shared high-fitness phenotype[42]. Evolutionary pressure to retain a stable phenotype can favour compensatory changes, but this pattern does not require compensatory evolution; alleles that have no effect when they arise may simply be incompatible with alternative genetic backgrounds. Divergence between lineages can thus draw entirely from substitutions that are neutral within lineages but incompatible across lineages[43]. From the perspective of an experimental geneticist, this model holds that suppressor alleles fix by chance before the alleles they suppress even arise, which renders both cryptic. This idea has been formalized in several models[44,45].

Modifiers of rare mutations. The second class of rarely-exposed G × G interactions — modifiers of rare mutations — also seems to be ubiquitous[46]. Mendelian genetic disorders in humans are, by definition, examples of rare mutations inducing phenotypes, but even diseases with simple genetic bases can present as complex physiological disorders, and differences across patients with the same disease-causing allele indicate that genetic background is important. For example, cystic fibrosis is one of the most common monogenic disorders in humans and is caused by recessive mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which affect the lungs, intestines, pancreas and metabolic homeostasis. Recently, several modifier loci that influence the expression of one or more of these physiological targets have been identified[47]. Similarly, a mouse model for congenital heart disease that is caused by mutations in the NK2 homeobox 5 (*Nkx2-5*) gene was used to map multiple modifiers that substantially affect risk[48]. These genetic modifiers have been explicitly defined using the same language as CGV, as loci that influence the action of a primary locus while remaining silent, or at least 'quiet', with respect to phenotype on their own[49].

Near-isogenic lines
(NILs). Inbred strains that are genetically identical to a progenitor strain except for a small region of the genome that is derived from a second strain.

Transgressive segregation
The appearance, in the progeny of a cross, of phenotypes outside the range of phenotypes that are present in the parental generation.

In experimental systems, mutation modifiers have been investigated in the context of genetic background effects. Evidence suggests that genetic background effects are pervasive[50]; occasionally, such effects have been exposed incidentally when evaluating the primary genetic defect. In *D. melanogaster*, the search for longevity-associated genes has revealed ubiquitous, and occasionally confounding, effects of genetic background on the expression of lifespan-mediating alleles[51,52]. In *C. elegans*, microevolution in the signalling network underlying vulva development indicates that genetic screens for vulva determinants will vary with the strain tested[22].

Only occasionally have these studies explicitly examined genomic background to test for CGV[2,53]. However, background effects compete in magnitude with the effect of mutations in the conventional genetic paradigm (that is, the effect of a mutation in a controlled genetic background). Moreover, background effects are themselves genetically complex, and modifiers of rare mutations themselves interact epistatically[54].

A particularly striking demonstration of the ubiquity of mutation-modifying background effects comes from a study in flies, in which mutations that affect startle behaviour in the Canton-S strain were introgressed into different wild-type backgrounds[55]. In each case, genetic background significantly influenced the effect of the mutation, and effects were smaller in wild-type backgrounds than in the Canton-S strain. The implication is that these mutations have strong effects in the background in which they were identified by phenotypic screens, whereas their effects in random backgrounds are lower and, in many cases, non-existent.

Box 3 | **Developmental system drift**

Cryptic genetic variation represents hidden polymorphism within populations, and developmental system drift (DSD) is hidden divergence among species. This phenomenon describes the divergence of genetic developmental mechanisms even as the phenotypic traits they determine remain static[106]. Evidence for DSD can be found in observations of species hybrids — in which morphological characteristics are malformed despite identical trait expression between the parental species[107] — as well as in the molecular divergence of conserved processes, including sex determination in Diptera[108].

A well-studied example of DSD is the evolution of the nematode vulva. Within the *Caenorhabditis* clade, species show conservation of signalling pathways and vulva organogenesis but substantial differences in the relative importance of signals for cell fate specification[26]. Basic morphology has also been conserved between *Caenorhabditis elegans* and the distantly related *Pristionchus pacificus*. In both species, the vulva is derived from the same cells through the same cellular processes[109], but its development is induced by different genetic mechanisms, principally epidermal growth factor signalling in *C. elegans* and Wnt signalling in *P. pacificus* (reviewed in REF. 110).

DSD can arise from both selection and neutral processes[26,111,112]. Natural selection might drive DSD by targeting pleiotropic alleles that are fully penetrant in one tissue but that act cryptically in others[26]. Gene network simulations confirm that selection on pleiotropic targets can lead to rapid evolution of DSD[113], and such a process makes sense for DSD of the nematode vulva because signals for vulva induction are known to mediate many other processes[114]. Indeed, this seems to be the case for a cryptic nucleotide for vulva cell fate specification that also affects egg laying and sperm production[62].

Genotype-by-environment interactions. The other basis for CGV, G × E interactions, is undoubtedly prevalent, as the expression of genotype routinely depends on the environment in all genetic systems[56–60]. The type of G × E interactions that underlies CGV is conditional neutrality, which is when genetic variation is less penetrant (or quieter) with respect to phenotype in one environment but more penetrant (or louder) in another. Studies that examine the genetics of local adaptation — most commonly conducted in plants, which are stationary with respect to their environment — report widespread findings of conditional neutrality[61].

The scenario of conditional neutrality raises a possibility that is not often invoked in the discussion of CGV: neutral alleles may be maintained in one environment by positive selection in, and subsequent migration from, another environment. An analogy to this is the case in which pleiotropic alleles are functionally neutral with regards to one trait but are targets of positive selection for another trait. For example, the *C. elegans nath-10* allele underlies CGV in vulva development but probably fixed in populations owing to increased egg laying[62]; whether this phenomenon is rare or common is completely unknown but, in theory, it may allow accumulation of alleles that are biased against deleterious fitness effects in the cryptic phenotype. Theoretical work that addressed niche adaptation across heterogeneous environments has shown how, across populations with gene flow, the effect of selection in marginal populations can be negligible[63] and that this is explicitly so for the case in which alleles are neutral in the predominant environment but not in the marginal environment[64]. Thus, it may be that CGV accumulates neutrally in populations because cryptic alleles are expressed at low rates, given that environments that reveal CGV are, by definition, rare. At the same time, if CGV is the result of adaptive canalization, with systemic buffering that breaks down when it would be adaptive to do so, then occasional exposure of the CGV in rare environments can create a pool of pre-adapted alleles in the event that the environment shifts to resemble the previously rare environments[65].

What role does CGV have in evolution?

For CGV to have an important role in evolution, there must be naturally occurring mechanisms that expose it. The influence of newly exposed CGV will strongly depend on its nature (FIG. 4). Does the CGV consist of damaging mutations that are concealed by buffering mechanisms? Is it disproportionately enriched for non-damaging mutations, given that they are, by definition, not unconditionally deleterious? Or are effects of the cryptic mutations completely random and perhaps symmetrically distributed? In the first case, exposure of the globally buffered variation under new conditions will invariably be deleterious, whereas release of specific subsets might produce novel beneficial phenotypes. In the second case, CGV will have a disproportionate role in adaptive evolution and will provide standing genetic variation to respond to selection. In the last case, revealed CGV

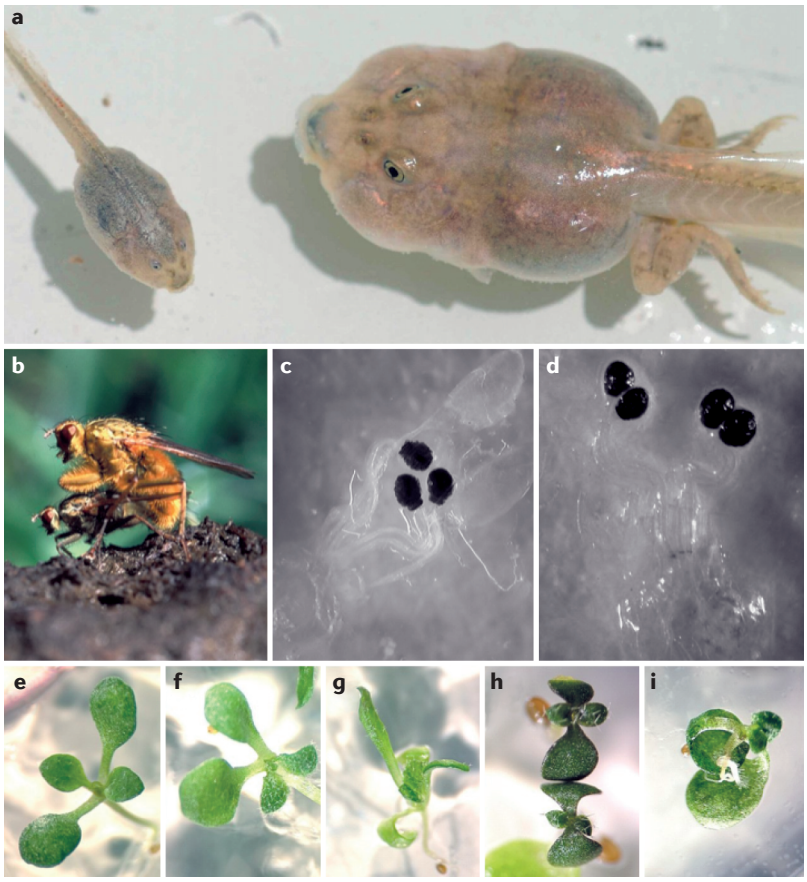

Figure 3 | **A sampling of experimental systems.** **a** | Spadefoot tadpoles are facultatively carnivorous; meat-eating tadpoles are larger and have shorter guts than their conspecifics that consume a plant-based diet. These two siblings are of the same age, but the tadpole on the left developed on a diet of plants and detritus. One study[31] fed a related species, the non-carnivorous *Scaphiopus couchii*, a shrimp diet and observed increased heritability for body size, developmental stage and gut length, which indicates that the dietary transition to the novel carnivorous feeding strategy in the spadefoot toad ancestor may have released cryptic genetic variation for these resource-use traits. **b–d** | Part **b** shows a mating pair of yellow dung flies. Female flies almost always have three spermathecae (that is, sperm storage compartments; part **c**). One study[29] perturbed spermathecae development by increasing rearing temperature to reveal cryptic genetic variation for four spermathecae (part **d**). **e–i** | One study[97] demonstrates that when *Arabidopsis thaliana* is exposed to the drug geldanamycin (GDA), which is an inhibitor of the heat shock chaperone protein HSP90, it shows a range of morphological abnormalities. Untreated, different accessions or varieties of the plant consistently develop into the wild-type phenotype (part **e**). Upon GDA treatment, different accessions showed abnormalities at varying frequencies. For example, the Shadara accession was most likely to show asymmetry in the arrangement of cotyledons (that is, embryonic leaves) and true leaves (part **f**), as well as deformed and radially symmetrical true leaves (part **g**). The Col accession more frequently gave rise to dwarf plants with dark, downward curling leaves (part **h**), and the *Ler* accession more frequently produced extremely curled immature stems (part **i**). Image in part **a** courtesy of D. Pfennig, University of North Carolina, Chapel Hill, USA. Image in part **b** courtesy of P. Jann, Natural History Museum Aargau, Switzerland. Image in part **c** courtesy of D. Berger, University of Zurich, Switzerland. Image in part **d** is modified, with permission, from REF. 29 © (2011) John Wiley and Sons. Images in parts **e–i** are modified, with permission, from REF. 97 © (2002) Macmillan Publishers Ltd. All rights reserved.

will increase genetic variance with beneficial and deleterious effects, and part of the population will be pre-adapted to new conditions and another part burdened with maladaptive phenotypes.

Environmental exposure of CGV. Stressful conditions are well suited to expose CGV, which facilitates adaptation to otherwise hostile environments[7]. However, there are substantive criticisms of this model, starting with the distinction between stressful conditions and novel conditions[66]. For CGV to accumulate neutrally, the conditions that expose it to selection must be rare in the history of the population. Stress is usefully defined as conditions that reduce fitness relative to that in the optimal realized environment of a population[67], and such stress is probably a typical experience for most populations. If particular stresses are routine, populations will adapt to them and evolve generalized buffering mechanisms, such as the Hsp90 system. If these mechanisms are particularly effective, then they may allow the fixation of mutations that are strongly deleterious when exposed by rarer or more extreme stresses[67]. If exposure is sufficiently rare (that is, occurring on the order of once during the expected coalescence time for neutral mutations), then fixation of conditionally lethal mutations may render CGV useless[68].

There are two models that use stress-induced loss of buffering as a mechanism for releasing useful variation. One model holds that buffering mechanisms are specific, such that particular conditions only expose subsets of the concealed CGV. This variation is then available, with limited undesirable pleiotropy, to selection that acts on specific traits[69]. An alternative is that the CGV-releasing conditions are rare but not exceedingly so, such that selection has an opportunity to purge the truly deleterious alleles, which leaves a residue of CGV that is depleted of disadvantageous variation and that harbours alleles at a mutation–selection-drift balance at frequencies determined by their effects integrated across the historical distribution of exposure[68].

Novel conditions do not need to be stressful. For example, a population introduced to an environment that lacks its competitors and predators and that contains new resources is novel but lacks stress, and CGV may cause some individuals to be better fitted to their new circumstances. Specific biotic and physical stresses are well studied, but how populations respond to novel environments remains poorly understood. A possible line of analysis focuses on CGV that is due not to evolved buffering mechanisms but to the inherent properties of molecular variants in biological networks. If CGV is due to a simple accumulation of conditionally silent variants under stabilizing selection in an ancestral environment, then a change of environment might increase variation symmetrically, which yields an increase in V_A without a change in mean phenotype. This new V_A is fuel for adaptation[70]. The increase in the proportion of the population that is far from the ancestral optimum, even without a change in the mean, is also a proposed mechanism for the increase in disease in human populations that have been exposed to the novel circumstances of modernity[1].

Genetic exposure of CGV. Although environmental changes can rapidly expose CGV owing to G × E interactions, the mechanisms responsible for releasing G × G variation are less obvious. Furthermore, although environmental changes can act on many individuals

simultaneously and instantly refigure the pattern of selectable variation in a population, epistasis depends on each individual's genetic constitution and affects them one at a time.

Models for the release of CGV stored by epistasis have focused on mechanisms that can radically alter genotypic frequencies across a population. Bottlenecks and founder events are natural candidates[71,72]. Although some theoretical work suggests that population contractions do not ordinarily release substantial V_A from epistasis[73,74], not all epistasis is equivalent. In particular, directional epistasis — whereby interaction effects tend to depart from additive effects in a consistent direction — can facilitate or thwart evolution, and increases in allele frequency at each locus systematically increase or decrease the marginal effects of one another[75,76]. Genetic data suggest that epistasis can be directional more often than not[55], and biochemical and gene-network models yield similar implications (reviewed in REF. 76). These results do not render a clear verdict on whether the patterns of directional epistasis are those that would tend to promote or hinder divergence, but empirical data provide weak support to an increase in V_A following bottlenecks[77,78].

Selection itself, by changing allele frequencies, is another mechanism that can expose CGV[79,80]. In an experimental dissection of loci that contribute to divergent evolution of chicken body weight under artificial selection[81], divergence was found to require a collection of epistatically interacting loci with effects that reinforced each other, which progressively exposed additional V_A during the course of the selection.

Although the contribution of epistatic CGV to adaptation remains somewhat unclear, the existence of abundant epistasis indicates that there are large numbers of silent polymorphic loci with the capacity, under specific conditions, to affect phenotypes. If the conditions induced by rare combinations of genotypes are also accessible to environmental perturbations, then this pool of G × G CGV may overlap G × E CGV. Environmental perturbations outside the organism will induce physiological responses, such as signalling cascades, that are mediated by the same factors which are vulnerable to genetic change within the cellular environment[66]. Furthermore, evolved buffering is expected to conceal both types of CGV by the same mechanisms[82].

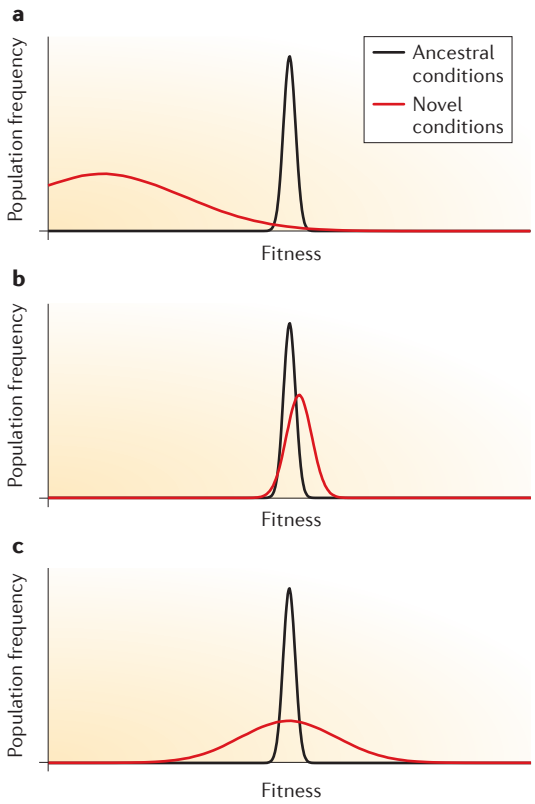

Figure 4 | **Fitness effect distribution of cryptic genetic variation in new conditions.** Three simple scenarios illustrate alternative outcomes for exposure of cryptic genetic variation (CGV). In black is the population's heritable variation in fitness (that is, the non-cryptic standing genetic variation in breeding values for a phenotype) under the normal condition; the transformed fitness distribution following a change of environment or genetic background is shown in red. **a** | Under a buffering scenario, a large proportion of the cryptic variants will be strongly damaging, and their exposure will primarily generate low-fitness phenotypes. **b** | Under an enrichment model, occasional exposure of CGV in the history of a population will 'weed out' the strongly deleterious alleles and leave the CGV pool enriched for variation that improves the population's fit to its environment. **c** | Under a symmetrical scenario, newly exposed CGV simply increases the heritable phenotypic variance around the same mean.

CGV in adaptation. Early concepts of CGV were explicitly founded on the notion that it might facilitate an alternative path to adaptation[9]. Under the most extreme scenario, CGV might underlie the evolution of novelty and major evolutionary transitions. Evolution of complex traits might require multiple changes, each of which is deleterious on its own and hence resistant to fixation. However, when individual alleles segregate neutrally, they may recombine into the same background to reach appreciable frequencies, and prerequisite alleles might even fix before they are revealed in the stimulus environment. This scenario provides a potential mechanism for circumventing low-fitness valleys in an adaptive landscape. At the same time, CGV should prove valuable in ordinary adaptive evolution, when changes in circumstances reposition a population on the flanks of a novel fitness peak. In such situations, CGV provides standing genetic variation for a rapid response to selection[83]. Whether CGV routinely contributes to either evolutionary scenario is an open empirical question, although data from experimental and natural settings are starting to shed some light on this issue.

CGV in adaptation:* in vitro *experiments. Two of the most definitive demonstrations that CGV can facilitate adaptation come from manipulations of *in vitro* populations of biological molecules. One study[84] evolved populations of ribozymes on a novel substrate. Populations

that had previously accumulated CGV under stabilizing selection on the ancestral substrate adapted more rapidly than populations that lacked CGV. The populations with CGV harboured genotypes that, although as fit as the wild type on the ancestral substrate, were also pre-adapted to an unseen substrate. In effect, the exploration of neutral genotypic space in one environment left these populations poised to adapt to an environment they had never 'seen'.

Similar work[85] demonstrated the same principle in a cytochrome protein; two cytochrome P450 molecules were mutagenized, and their activities on novel substrates were tested. The two starting molecules shared function on their initial substrate, but one was an evolved variant of the other and differed by eight amino acid residues that were fixed by selection for thermostability. Following mutagenesis, the highly thermostable molecule better retained its ability to fold, which in turn permitted activity on the novel substrates[85]; that is, newly beneficial mutations that were accessible to the thermostable P450 molecule were inaccessible to the ancestor owing to epistasis with the stability-conferring mutations. Ancestral CGV in thermostability would therefore facilitate adaptation to novel environments. These experiments show compelling evidence for the adaptive potential of CGV, although their applicability to *in vivo* systems, specifically those with recombination, is currently unclear[86].

CGV in adaptation:* in vivo *experiments. Few demonstrations of CGV-mediated adaptation surpass Waddington's original genetic assimilation experiment: selection on heat- or ether-induced phenotypes. More recently, various studies in *D. melanogaster* and *A. thaliana* have shown that selection on phenotypic variation revealed by Hsp90 depletion also yields responses (BOX 1). In yeast, the release of CGV by inhibiting Hsp90 activity showed substantial variation in growth rates across many environments and increased fitness in some cases. A similar result was achieved under high temperature stress, which may deplete the Hsp90 folding reservoir and offers a potential mechanism by which natural populations both maintain robust phenotypes and facilitate rapid adaptation in new environments[87].

Evolved plasticity can also draw on CGV[88,89]. An experimental test of this scenario[90] selected for a temperature-dependent larval colour polyphenism in the hornworm *Manduca sexta*, which produces larvae with colour that is insensitive to the ordinary range of developmental temperatures. The researchers used acute heat shock to expose CGV for larval colour; after 13 generations of selection on heat shock-exposed CGV, they had evolved a polyphenic line that showed larval colour with a switch-like dependence on temperature within its ordinary range. This polyphenism matches a naturally occurring, and putatively adaptive, one in the related species *Manduca quinquemaculata*.

Polyphenism
The phenomenon whereby a single genotype produces multiple discrete phenotypic states under different conditions.

CGV in adaptation: evidence in nature. Going beyond the laboratory and into the field, the gaps in evidence of the role of CGV in adaptation become apparent[67]. However, several studies provide compelling demonstrations of how ecologically relevant conditions might have facilitated adaptive change. Oceanic sticklebacks that were reared in low salinity showed marked increases in V_A for body size, which indicates that CGV in ancestral oceanic populations may have facilitated the adaptive evolution of smaller size in freshwater habitats[28]. Similarly, surface fish reared in low-conductivity water that mimicked cave conditions showed increased variation for eye size that may underlie the adaptive morphology of blind cavefish[91]. Spadefoot toads have a novel feeding strategy of facultative carnivory that is accompanied by a derived body morphology. When a related species that stands as a proxy for the spadefoot ancestor was fed a carnivorous diet, it showed increased heritability for body size, developmental stage and gut length, which indicates that diet may have released adaptive morphological variation[31] (FIG. 3).

A striking suggestion of the direct role of CGV in phenotypic evolution comes from a study of the genetic origins of domesticated maize, which is the product of centuries of artificial selection. CGV for seven traits in teosinte — the ancestor of domesticated maize — was observed in crosses between heterogeneous teosinte and a single inbred strain of maize[92]. The genetic contribution of the inbred maize strain acted as a genomic perturbation to the teosinte genotypes, which were the only source of genetic variation in the experiment. Although the pure teosinte strains were phenotypically invariant, substantial variation in traits that relate to branch and inflorescence morphology was released in the test cross. QTL mapping identified multiple causal regions and provided some evidence that loci that were already identified to account for phenotypic differences between teosinte and maize harbour CGV for the same traits in teosinte.

Given the number of high-confidence findings about CGV — its abundance in populations, it potential, in theory, to fuel evolution and its demonstrated ability to do so in experimental settings — the paucity of evidence for its role in adaptation in natural populations is striking. However, well-understood examples of the genetic basis of adaptive evolution are scarce in general, and the higher evidential threshold required by CGV — the demonstration that phenotypic effects of alleles are conditional on ancestral and derived circumstances — makes the task daunting.

CGV and complex human disease. An alternative to the role of CGV in adaptation is its role in disease. Alleles that accumulate while hidden may have an important role in the emergence of complex human diseases, although there is currently limited empirical evidence for this hypothesis. The recent move of human populations into novel conditions — including changes to hygiene, diet, and exposure to environmental insults (for example, tobacco and industrial pollutants) and new pathogens (for example, HIV) — is hypothesized to have revealed pre-existing allelic variation for modern disease susceptibility[93]. These alleles may have

accumulated by phenotypic canalization on fundamental aspects of mammalian physiology and are exposed by previously unseen conditions[1]. For example, body mass index (BMI) is associated with many modern diseases[94] and has shown a substantial global increase in the last century[95]. Alleles that influence BMI, as well as alleles with BMI-dependent effects, are likely to have changed in their contributions to phenotypes in contemporary human populations, which potentially increases the genetic variance. Further tentative support for this scenario is the observation that ancestral (as opposed to novel) alleles underlie disease susceptibility for multiple common diseases[1]. The question that demands testing is therefore whether the environmental and cultural conditions that are associated with modern complex diseases actually increase heritability by exposing cryptic genetic variance.

Conclusions

More than 70 years after the recognition that populations harbour a cryptic store of standing genetic variation, the nature and importance of this CGV is better understood in theory[3,68] than in nature. Nevertheless, some facts are clearly established. CGV, which is concealed by G × G and G × E interactions, is abundant in natural populations and can be released under novel conditions. Such variation has the potential to fuel a selective response and represents at least crouching variation if not standing genetic variation[96]. Moreover, although CGV is connected to hotly debated topics such as capacitance, robustness and canalization, its study is separable from those issues and its occurrence is not dependent on them.

One of the key questions for the future concerns the extent to which CGV in natural populations is shaped by selection. Our classical definition of cryptic genetic variants supposes that these alleles accumulate in populations under strict neutrality and are never tested by selection. Quantitative and population genetic viewpoints argue that this definition may be too strict. Alleles with contributions to V_A that increase under novel conditions may constitute a more useful class, as these variants will have been filtered of the ones that are strictly deleterious when exposed. The challenge then is to construct a more continuous account of conditional variation that integrates both the degree of condition-dependence of effect sizes and the frequency distribution of conditions. This frequency distribution is a question that demands ecological investigations outside the normal ken of molecular geneticists.

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Competing interests statement
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