FUNDAMENTAL CONCEPTS IN GENETICS

The genetics of inbreeding depression

Deborah Charlesworth and John H. Willis‡*

Abstract | Inbreeding depression — the reduced survival and fertility of offspring of related individuals — occurs in wild animal and plant populations as well as in humans, indicating that genetic variation in fitness traits exists in natural populations. Inbreeding depression is important in the evolution of outcrossing mating systems and, because intercrossing inbred strains improves yield (heterosis), which is important in crop breeding, the genetic basis of these effects has been debated since the early twentieth century. Classical genetic studies and modern molecular evolutionary approaches now suggest that inbreeding depression and heterosis are predominantly caused by the presence of recessive deleterious mutations in populations.

Fitness-related characters
Survival, growth rate and fertility.

Inbreeding coefficient
The probability that two alleles in an individual were both descended from a single allele in an ancestor (that is, that they are 'identical by descent').

Mutation–selection balance
The balance between mutations that introduce deleterious alleles into the population and the removal of such alleles by natural selection. The result is that such mutations are present at low frequencies but, despite selection, are never entirely absent.

**Institute for Evolutionary Biology, Ashworth Laboratories, King's Buildings, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK.*
‡Department of Biology, Box 90338, 3314 French Family Science Center, Science Drive, Duke University, Durham, North Carolina 27708, USA.
Correspondence to D.C.
e-mail: deborah.charlesworth@ed.ac.uk
doi:10.1038/nrg2664

The harmful effects of inbreeding were first documented in detail and quantified by Charles Darwin, who carried out experiments on 57 plant species that involved self-fertilization and outcrossing between unrelated individuals[1]. Darwin's laborious study was motivated by the desire to explain why reproduction by outcrossing is prevalent in nature, what maintains the sometimes complex outcrossing mechanisms of flowers[2], and why numerous plant species have systems that prevent self-fertilization[3]. Darwin's experiments supported his hypothesis that self-fertilization must be strongly disadvantageous for the progeny produced — it lowered vigour and fertility in most of his study species[1].

Inbreeding lowers fitness-related characters in many species of plants and animals, including humans (TABLE 1 lists some non-human examples). Major abnormalities are more frequent in inbred (consanguineous) families than in outcrosses. These abnormalities include mutant phenotypes that are lethal early in life, such as chlorophyll-deficient albino seedlings in plants and developmental defects in fish[4], or genetic diseases in humans. Even when there are no overt major abnormalities, inbreeding depression is detectable by the lower fertility, survival and growth rates of individuals with high inbreeding coefficients (BOX 1; TABLE 1).

The survival and fertility of individuals in experimentally produced inbred lines is frequently so low that many such lines go extinct. When surviving lines are intercrossed, the 'hybrids' often have higher quality than their inbred parents and frequently exceed the best parent values for several characters[5]. This increased performance of first filial generation (F_1) hybrids is called heterosis, or hybrid vigour. In many crops, heterosis has been extensively studied for characteristics of economic interest, such as drought tolerance, disease resistance and yield, and some of these characteristics may also be important for fitness in natural populations[6].

It is important to understand the genetic basis of these effects. Inbreeding depression is caused by increased homozygosity of individuals, as shown in FIG. 1. There are two genetically distinct ways in which increased homozygosity can lower fitness: increased homozygosity for partially recessive detrimental mutations and increased homozygosity for alleles at loci with heterozygote advantage ('overdominance'). Deleterious alleles will generally be present in populations at low frequencies (mutation–selection balance), whereas overdominant alleles at a locus are maintained at intermediate frequencies by balancing selection (BOX 2).

Because both genetic mechanisms might also cause heterosis when inbred lines are intercrossed (BOX 2; TABLE 2), understanding the underlying genetics is crucial for crop breeders. If loci with heterozygote advantage are common (BOX 3), artificial selection in agricultural species should, at least in part, select for strains that manifest substantial heterosis. Breeding crop plants with a uniform, highly heterozygous genotype with high fitness could also be desirable, and could perhaps be achieved by asexual seed production[7]. If, however, heterosis in crops is mainly due to detrimental mutations, it is potentially possible to 'purge' these alleles to produce high-yield mutant-free strains. Understanding the genetic basis of inbreeding depression can also help to answer the long-debated question in evolutionary biology of why genetic variation in fitness-related characteristics exists in so many species, including humans[8].

Table 1 | **Detecting inbreeding depression and genetic load**

Type of organism	Method	Quantities estimated	Refs
Direct detection (experiments or pedigree information)			
Self-compatible hermaphrodite animal or plant (or monoecious plant*)	Comparisons of offspring produced by self-fertilization with offspring produced by outcrossing	δ‡ for one generation of selfing	1,96
Cyclically asexual animals§	Mating females with their brothers from the same asexual clone; this is genetically equivalent to self-fertilization	δ for one generation of selfing	19
Organisms with separate sexes	Sib-mating for one or more generations and other types of mating to produce experimental individuals with various inbreeding coefficients	Inbreeding load	140,141
Haplodiploid organisms	Mother–son inbreeding (generating higher inbreeding coefficients than ordinary diploid sib-matings)	None (detection only)	142
Ferns or mosses	Intragametophyte selfing‖	Effect of complete genome homozygosity	143,144
All organisms	Analyses of the relationship between trait values and inbreeding coefficients based on pedigree information	Inbreeding load	145
Indirect detection (using genetic markers)			
All organisms	Use of inbreeding coefficients estimated from frequencies of homozygotes and heterozygotes for genetic markers or SNPs	Inbreeding load	146–149
All organisms	Examination of genetic ratios at marker loci: deficiency of one homozygote in families or a significant heterozygote excess in a family (or in inbred lines relative to the heterozygote frequency predicted for neutral alleles by the inbreeding coefficient) suggests inbreeding depression due to identity-by-descent for a gene linked to the marker	Detection; estimates of selection and dominance coefficients	10, 150–153
Cyclical parthenogens§	Detection of an increase in heterozygote frequency over asexual generations	None (detection only)	154

Inbreeding depression can be detected by direct experiments or by detecting the effects of inbreeding indirectly using genetic markers. Many of the approaches listed in the table detect the ill effects of inbreeding (that is, they reveal 'inbreeding load') but do not quantify the load or inbreeding depression. Quantification of both the degree of inbreeding and its effects are necessary to evaluate whether a given genetic model can explain the magnitude of the effects observed and to compare the magnitude of effects between species or experiments. Approaches that provide quantitative estimates of inbreeding depression are therefore noted. In natural population studies, measures for individual characters are often multiplied to estimate overall fitness (assuming that genetic effects in different life stages act independently). *A plant that has separate male and female flowers. ‡δ is a measure of the reduction in fitness trait values (BOX 1). §Cyclical parthenogenesis is the alternation, over the year, of sexual and asexual reproduction, with the latter leading to the production of genetically identical males and females that can mate with one another. ‖The fertilization of female gametes that are produced by a haploid gametophyte (by mitosis) by male gametes that are produced by the same gametophyte, which yields completely homozygous zygotes.

We begin by describing evidence showing that natural populations contain deleterious mutations (mutational load) and then explain how several arguments based on studies of heterosis, including gene expression studies, that seemingly support a major role for overdominance are also readily explicable by deleterious mutations. We then describe how molecular evolutionary studies and fine mapping of genes involved in fitness variation support the view that inbreeding depression is predominantly caused by the cumulative effects of deleterious mutations at many loci, probably with a contribution from overdominance at a few loci.

The evidence for deleterious recessive mutations

Both inbreeding depression due to increased homozygosity and the benefits of intercrossing (heterosis) arise from the generally lower fitness of one or both homozygotes compared with the heterozygotes. The genetic explanations for both (BOX 2) therefore involve the dominance levels of the alleles — they either hypothesize overdominance (heterozygote advantage), or partially or entirely recessive mutant alleles lowering homozygous fitness (FIG. 1). It is difficult to quantify the different genetic contributions to inbreeding depression or heterosis because many proposed tests (see below) cannot distinguish between linked deleterious mutations in a genome region and a single locus with heterozygote advantage (FIG. 1).

The genetic causes of inbreeding depression are therefore studied by integrating genetic and population results from genetically tractable organisms rather than from humans, despite the interest in understanding the genetic basis of human genetic variation for fitness and inbreeding effects (BOX 1). One helpful approach is to test whether mutations can account for the observed extent of inbreeding depression. Therefore, we first review evidence that mutations that are deleterious for fitness are invariably found in populations whenever they have been tested for.

***Rare recessive lethals in natural populations of* Drosophila melanogaster.** Deleterious mutations are certainly present in natural populations. In experiments that used balancer stocks to make entire chromosomes homozygous to test them for viability, natural *Drosophila melanogaster* populations were shown to carry many individually rare, highly recessive and highly detrimental mutations[9], which is consistent with mutation–selection balance. About 30% of the wild-type second or third chromosomes isolated from males sampled in the wild are lethal when homozygous[9]. Heterozygotes for lethal chromosomes isolated from different wild individuals are almost always viable, which

Balancing selection
Selection — such as heterozygote advantage and frequency-dependent selection — that maintains genetic variants in a population.

Purging
Reducing the frequencies of deleterious mutations in inbred populations, thereby lowering the mutational load (the presence of deleterious mutations in populations).

Balancer stock
A strain of fly that contains a chromosome with genetic markers and with an inversion to prevent recombination with other arrangements. Such chromosomes are used to breed stocks with 'extracted' wild-type chromosomes for estimates of homozygous and heterozygous effects.

Box 1 | How inbreeding depression is detected and measured

In humans, consanguinity adversely affects health; this effect is often slight, however, because the low degree of inbreeding in humans leads to weak effects[57]. Despite inbred individuals occasionally performing better — for example, Icelandic families that are more closely related than second cousins have more offspring[91] — overall they show fitness reductions, including increased stillbirth and infant death, birth defects[92–94], and probably lowered post-reproductive health[95]. Inbreeding depression often affects fertility[44,96,97].

Inbreeding depression is usually studied in organisms that can self-fertilize, such as self-compatible plants or hermaphrodite animals, because here intense inbreeding has the strongest effects. Even a single generation of selfing often more than halves measures of lifetime fitness in normally outbreeding plants[27,29,98] and animals[99]. Inbreeding over multiple generations, including milder inbreeding, such as sib-mating in mice or *Drosophila melanogaster*, almost always reduces fitness compared with the outbred initial population. Outbreeding *Caenorhabditis* species suffer severe inbreeding depression[100], and multiple generations of forced inbreeding failed to make some parts of the genome homozygous, probably because these regions carry deleterious recessive mutations[10].

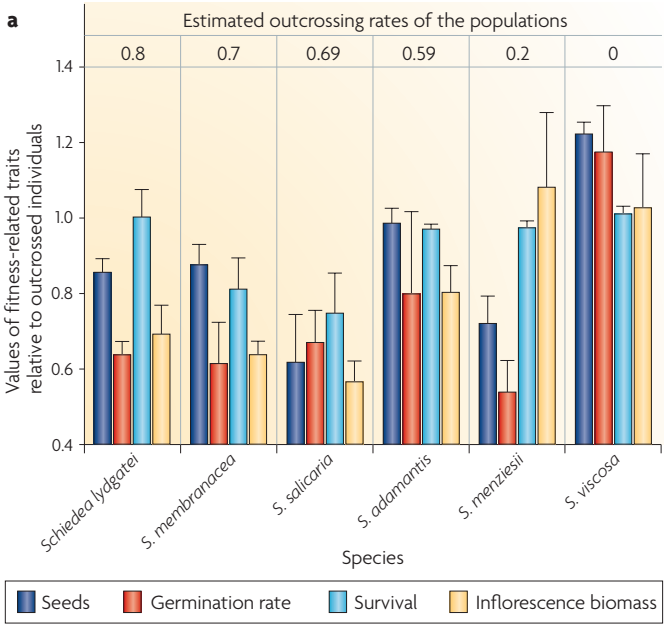
Detecting inbreeding depression in self-fertile species

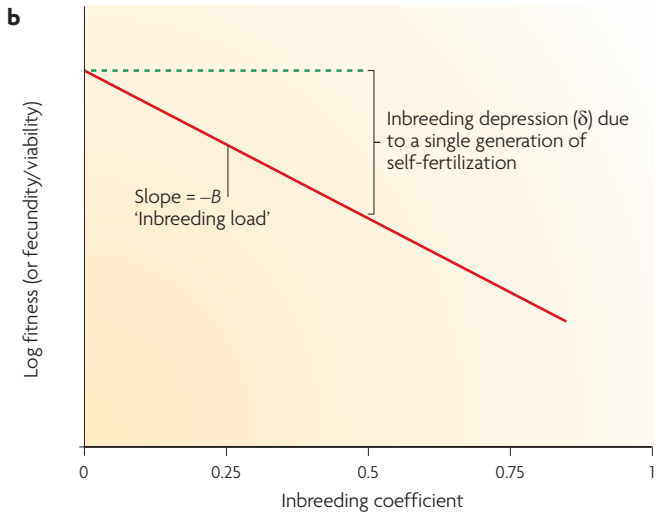
In self-fertile organisms, such as plants and some hermaphrodite animals (for example, snails), inbreeding depression can simply be studied by measuring the survival and/or reproduction of individuals after a single generation of self-fertilization, as in Darwin's experiments (TABLE 1). Seeds are generated by self-fertilization and by outcrossing, and are planted together in paired trials in the same pots in an effort to equalize differences in the growth environment[1]. The offspring are evaluated for traits that are closely related to what is now called Darwinian fitness: survival from the zygote or seed stage to some convenient later life stage, or fertility characters. The reduced values of such traits are often quantified by $\delta = (w_X - w_S)/w_X$, in which w_X and w_S are, respectively, the values for outcrossed progeny and progeny produced by self-fertilization. In the figure, part **a** shows an example of the variation in fitness-related traits among species that have different outcrossing rates: inbreeding depression was estimated using self-fertilization of hermaphrodite individuals in six species of the flowering plant genus *Schiedea*[101]. The figure shows the tendency for less inbreeding depression in the species with more inbreeding, as predicted (see main text).

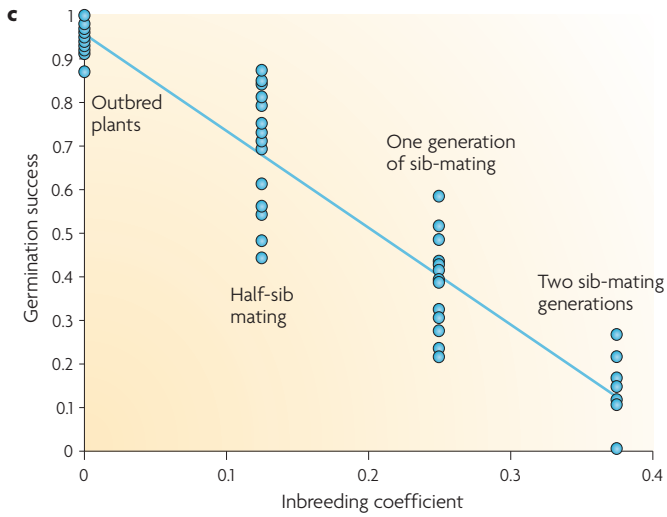
Detecting inbreeding depression in outcrossing species

In outcrossing species, including humans, the fitness traits of outcrossed individuals can be compared with those of individuals that have been inbred to different known degrees, measured by the inbreeding coefficient. The 'inbreeding load', *B*, is defined as the rate at which fitness declines with increased inbreeding coefficient (which determines the proportion of homozygous loci in the genome). In the figure, part **b** shows the theoretical fitness decline (measured as the regression slope on a logarithmic scale) with increased inbreeding coefficient; a single generation of self-fertilization gives progeny with an inbreeding coefficient of 0.5, assuming that the parents are unrelated (that is, they have an inbreeding coefficient of 0). Part **c** shows the observed linear decline in a fitness-related character in a sib-mating experiment in the outcrossing plant *Silene latifolia* (white campion)[102].

Quantifying the load allows tests of the prediction that inbreeding populations will generally show little decline in fitness with inbreeding (due to purging, see main text). Note that a given fitness difference between selfed and outcrossed progeny in an outcrossing population implies a higher inbreeding load than the same fitness difference between selfed and outcrossed progeny in an inbreeding population. This is because the progeny inbreeding coefficients being compared are 0–0.5 for the outbreeder, versus a much higher inbreeding coefficient for the inbreeder (for the inbreeder, the initial inbreeding coefficient is almost 1, versus 0 for its outbred progeny). Part **a** is modified, with permission, from REF. 101 © (2005) Wiley. Part **c** is reproduced, with permission, from REF. 102 © (2000) University of Chicago Press.







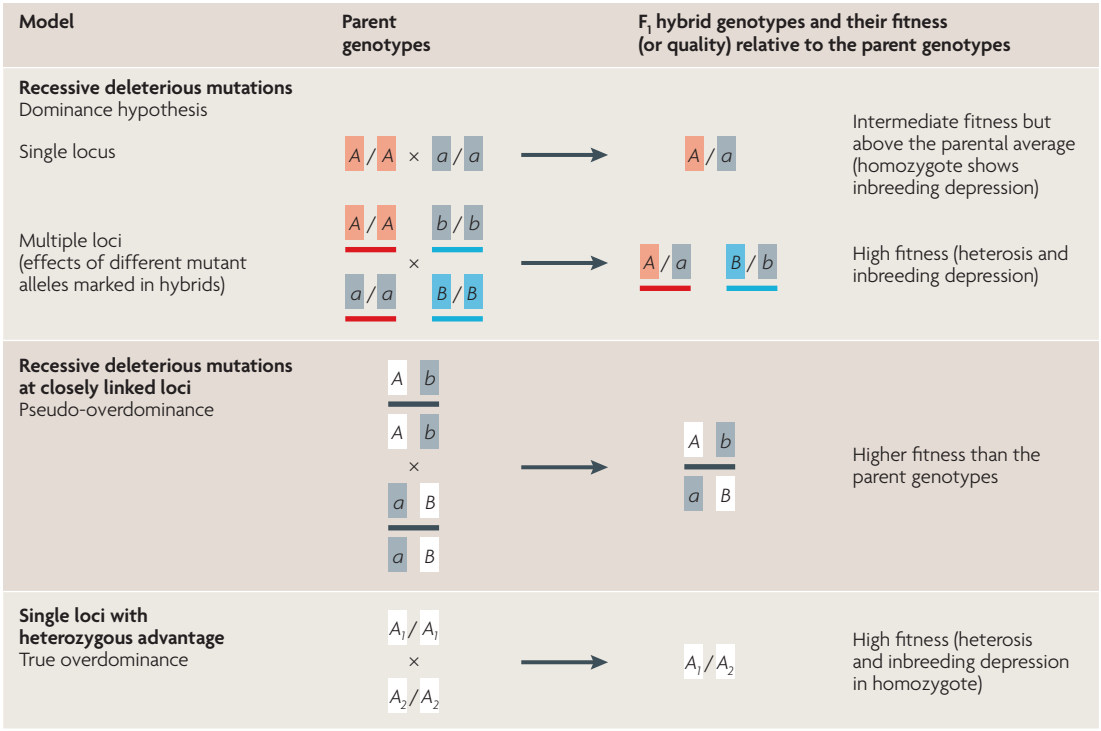


Figure 1 | **Summary of the main genetic hypotheses for inbreeding depression.** These hypotheses were developed by maize geneticists early in the twentieth century but have proved difficult to test (see main text). The increased homozygosity of inbred individuals can lower fitness either because of deleterious mutations with recessive effects, which cause homozygotes to have lower survival or fertility (top and middle rows), or because loci exist with different alleles that result in the higher fitness of heterozygotes (overdominance, bottom row). For the dominance and pseudo-overdominance (mutational) hypotheses, the figure shows how the higher homozygote frequencies for recessive deleterious mutant alleles (indicated as *a* and *b*) among inbred individuals will cause lower fitness than in more heterozygous outbred individuals or hybrids. In the overdominance hypothesis, inbred individuals are less likely to be heterozygous for the two alleles (A_1/A_2) than outbred individuals or hybrids and therefore have lower fitness. See BOX 2 for a more detailed explanation of these hypotheses.

shows that the mutations are recessive. Different wild lethal chromosomes carry different lethal mutations (they map to many different single loci); if balancing selection, such as heterozygote advantage, were important, the alleles that cause low homozygote fitness should be at intermediate (rather than low) frequencies and would often be shared between different individuals.

Unlike the autosomes, X chromosomes from wild fly populations are rarely homozygous lethal. This also supports mutation–selection balance, because males are XY: the *D. melanogaster* Y chromosome lacks nearly all of the genes that are present on the X chromosome, so X-linked lethal mutations kill their male carriers and are quickly lost from populations. Similarly, in outcrossing nematodes, most of the X chromosome can be made homozygous after inbreeding, unlike the other chromosomes[10].

Do the 70% of *D. melanogaster* chromosomes that are non-lethal when homozygous carry milder deleterious mutations? Their effects on viability can be estimated in balancer chromosome experiments by comparing the adult ratios of wild-type homozygotes and their heterozygotes with the balancer. Lower than Mendelian frequencies of homozygous flies show that homozygosity usually lowers survival to the adult stage[9], revealing the presence of minor-effect variants in populations. Quantitative experiments that competed individual non-lethal second chromosomes against balancer chromosomes in laboratory fly populations[11,12] have shown that whole chromosomal homozygosity in *D. melanogaster* reduces fitness over the whole lifetime by about 84% on average.

***Visible lethal and sterility mutations in plants and animals other than* D. melanogaster.** In most species other than *D. melanogaster*, it is unknown how much inbreeding depression is due to lethal mutations, because homozygous embryos often die during the earliest stages of development, which makes such mutations difficult to detect. However, embryonic lethals that cause visible developmental defects can be studied in offspring from controlled inbred crosses. By self-fertilizing (selfing) *Mimulus guttatus* plants collected from the wild and examining large numbers of progeny, it was found that 2.4–6.5% of plants from natural populations were heterozygous carriers of recessive chlorophyll-deficient lethal mutations[13]. Similar results were found in natural mangrove tree populations[14] and in sib-mated buckwheat[15,16]. The genetic factors present in *M. guttatus* and buckwheat were tested for allelism by intercrossing heterozygotes, and all of the lethal alleles that were

Hermaphrodite
An individual with both male and female reproductive functions (including monoecious plants, which have separate male and female flowers).

Darwinian fitness
Survival from zygote to maturity (viability) and reproductive performance (fertility); often measured as the product of viability and fertility measures.

Box 2 | Genetic hypotheses for inbreeding depression and heterosis

The main genetic hypotheses for inbreeding depression (FIG. 1) fall into single-locus explanations and hypotheses involving more than one locus.

Single-locus hypotheses

Detrimental mutant alleles that are largely recessive and are present at low frequencies in populations can contribute to inbreeding depression because homozygotes are rare except after inbreeding. This hypothesis (FIG. 1, top row) is often called 'the dominance model'. Heterozygotes for a loss-of-function or large-effect mutation indeed often have almost the same level of function as wild-type homozygotes ('directional dominance'). Mildly deleterious mutations, however, are often only partially recessive, so heterozygote fitness is only ~5–25% higher than the homozygote average[103]. Each such mutation contributes little to inbreeding depression, although these mutations are abundant in populations and in aggregate their effects may be large.

In contrast, overdominant alleles (FIG. 1, bottom row) are maintained by balancing selection, often at intermediate frequencies in populations, even if one or both homozygotes have low fitness. Balancing selection also sometimes maintains chromosomal inversion polymorphisms[104] and polymorphisms for other large genome regions with suppressed recombination (including the meiotic drive regions of chromosomes[105]). These may accumulate different mutations that lower fitness when homozygous, making the region overdominant[106]. Claims of single-locus overdominance (for example, REF. 107) should therefore exclude inversions. In *Mimulus guttatus*, much of the inbreeding depression for male fertility is caused by polymorphic chromosomal rearrangements that are associated with recessive partial male sterility and meiotic drive[108,109].

Other forms of balancing selection also maintain genetic variation, but probably rarely contribute to inbreeding depression. For instance, recessive alleles with harmful effects on a fitness trait may be common in populations because of their beneficial effects on other traits or life stages or in other environments (antagonistic pleiotropy), but dominant alleles are unlikely to always give higher fitness[44].

Two or more loci

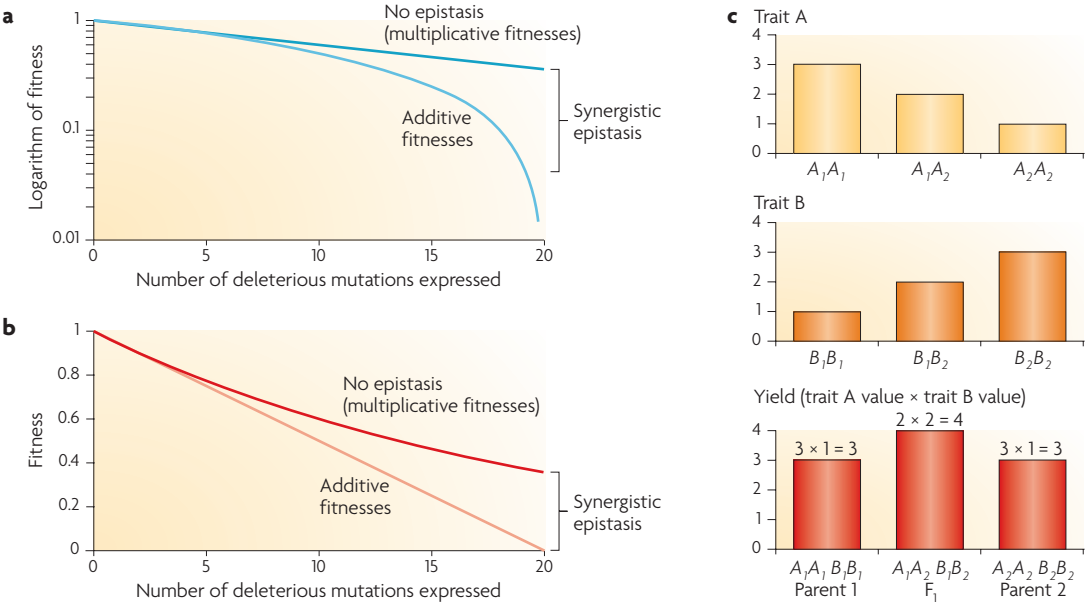
Two very different types of situations that involve multiple loci are important for understanding inbreeding depression.

Pseudo-overdominance may often underlie inbreeding depression and heterosis. Complementation can occur between unlinked deleterious alleles in a hybrid, producing heterosis (FIG. 1, top row). Similarly, a genome region may contain two or more closely linked genes, with each parental chromosome having different deleterious recessive alleles in repulsion (FIG. 1, middle row). Homozygotes for the chromosomal region will suffer reduced performance, which will produce apparently overdominant factors in quantitative trait locus studies, even though two distinct loci are actually involved.

Multiplicative and non-multiplicative interactions

If many moderately or slightly deleterious alleles are present in an outbred population, the numbers that are homozygous in an inbred genotype will determine its fitness. Different genes can interact in many possible ways, but often the fitness-reducing effects of homozygosity for deleterious alleles (mutant alleles or alleles at loci with overdominance) act roughly multiplicatively. This will occur when different deleterious mutations affect the trait independently. In the figure below, part **a** shows how the scale on which the trait is measured can affect whether epistasis is detected. Multiplicative effects result in a linear decline on a logarithmic scale (therefore this scale is used in part **b** in the figure in BOX 1), whereas additive effects produce epistasis of the synergistic form (with increasingly severe losses of fitness as the number of homozygous alleles increases; see part **a**). If mutations reduce fitness more than additively, synergism can be even stronger.

Although individual loci with completely additive alleles (no dominance) will not contribute to inbreeding depression, two or more such loci can cause heterosis when they affect a trait, such as yield or fitness, that results from the multiplicative combination of component traits[110,111]. In the figure, part **c** shows an example: total seed number is the product of flower number (trait A, controlled by locus *A*) and seeds produced per flower (trait B, controlled by locus *B*).



Inversion
Rearrangement in which part of a chromosome is inverted in order with respect to a homologous chromosome in the same species or in a different species.

Meiotic drive regions
Regions containing genes that have non-Mendelian segregation in heterozygotes because one allelic version of the region is rendered non-functional during meiosis.

Complementation
Restoration of function in heterozygotes for two genes with recessive loss-of-function mutations (unless both mutations are in the *trans* configuration in the same gene, so that neither allele is functional).

Table 2 | **Differences between inbreeding depression and heterosis**

Nature of the difference	Inbreeding depression	Heterosis*
Genetic variation	Must be present within the species or population	Can appear in F_1 individuals between genetically uniform populations or strains
Effect of genetic drift in small populations	Lowers inbreeding depression due to mildly deleterious mutations in small populations	Heterosis due to mildly deleterious mutations is highest for small populations or highly inbreeding populations
Likelihood of outbreeding depression and its consequences	Unlikely without strong isolation or local adaptation, and therefore unlikely to affect the magnitude of inbreeding depression within a population	May lower the magnitude of heterosis
Complementary interactions between different deleterious recessive mutations	Can cause inbreeding depression if loci are linked, so homozygosity for the genome region lowers fitness (pseudo-overdominance, see FIG. 1)	Can cause heterosis even if loci are unlinked and even if heterozygous alleles at the loci cause phenotypes that are between those of the homozygotes (FIG. 2c)

*See BOX 3.

detected were again found to be at a low frequency[15,16] (as in *D. melanogaster*). In zebrafish and killifish, sibmated families often segregated for recessive alleles that were lethal during egg development[4], and the numbers of heterozygous lethal mutations were estimated to be similar to those in *D. melanogaster*.

Mutations that act later in the life cycle, including sterility mutations, can also be detected. In outcrossing plants, inbreeding experiments commonly reveal carriers for recessive male sterility mutations, and crossing experiments typically indicate that these alleles are individually rare[17].

Purging mutant alleles: indirect evidence for deleterious mutations. As already mentioned, if rare, highly deleterious recessive mutations cause a major portion of inbreeding depression, inbreeding should rapidly purge these mutations from a population or species. In haplodiploid organisms, models of purging predict that inbreeding depression due to deleterious mutations should be slight because recessive mutant alleles will be selected out in each generation of haploid males[18]. Inbreeding depression is indeed weaker in haplodiploids than in comparable diploids[19].

We have already mentioned Darwin's prediction that inbreeding depression favours the evolution of outcrossing[3]. Outcrossing is puzzling: compared with self-fertilization or asexual reproduction, it carries the risk of reproductive failure when potential mates are scarce, and it seems to waste the resources and time of organisms in terms of finding and attracting mates (or, in plants, attracting pollinators). Self-fertilization also has a strong genetic advantage because an individual contributes two copies of its genes to each progeny produced by self-fertilization, versus just one copy to each outbred progeny[20,21]. The intuitively appealing view that outcrossing is beneficial to populations — because the higher variability of outcrossing populations compared with inbreeding populations might allow the former to evolve in response to changes in their environments[22] — is no longer accepted, because the long-term advantages to populations cannot prevent inbreeding evolving, owing to the strong immediate advantages to individuals of self-fertilization.

Many theoretical studies have confirmed Darwin's idea that strong inbreeding depression can overwhelm the ecological and genetic advantages of self-fertilization[23]. The underlying genetics of inbreeding depression can produce different evolutionary outcomes. If it is mainly caused by recessive deleterious mutations, inbreeding in small populations may cause purging, which may allow selfing to become evolutionarily favoured[24]. In contrast, inbreeding depression due to heterozygote advantage can increase with greater inbreeding in a population, which will potentially favour the evolution of mating systems with mixed outcrossing and selfing[25] (although such mixed mating can also evolve for other reasons[26]).

Comparisons of inbreeding depression between naturally inbreeding species and naturally outcrossing species suggest modest purging (BOX 1), although some inbreeding depression is detectable in inbreeding species[27–29]. The mild inbreeding depression and heterosis that are commonly observed in highly selfing wild species[30] are therefore explicable as unpurged mutational load. However, this is not conclusive evidence: in highly inbreeding populations, overdominant alleles are not maintained (selection favours the better homozygote[31]), so both genetic hypotheses predict low inbreeding depression in such populations.

Contributions of mildly deleterious mutations. Theoretical models predict that purging is most effective and will happen especially quickly for large-effect mutations, and that inbreeding depression caused by cumulative effects of many individually rare, mildly deleterious mutations will not easily be purged by inbreeding[32,33]. We have already mentioned experiments that indicate that *D. melanogaster* populations carry mildly deleterious mutations, as well as lethal mutations. The differential purging of major and minor mutations might help in assessing their relative contributions to inbreeding depression.

Large sets of individuals from outcrossing populations can be inbred for multiple generations to eliminate major recessive mutations. The remaining unrelated inbred individuals can be inter-mated, and the inbreeding depression and fitness in this new outbred population can be compared with that of the ancestral population. In the plant *M. guttatus*, such an experiment purged little of the inbreeding depression, which suggests a minor role for large-effect mutations[34]. In the beetle *Stator limbatus*, however, inbreeding depression decreased by 35% to 73% in two replicate purged populations, which suggests a bigger contribution of large-effect mutations[35].

Haplodiploidy
The system in Hymenoptera (bees, wasps and their relatives) in which fertilized eggs develop into females and unfertilized eggs develop into (haploid) males.

Large-effect mutations
In the context of this Review, mutations that cause major phenotypic abnormality, disease, lethality or sterility.

Box 3 | **Heterosis and outbreeding depression**

Heterosis (or hybrid vigour, when the parents are different species) is often measured as the difference between the fitness traits of the first filial (F_1) generation and the best parent value. Heterosis and inbreeding depression can be caused by either of the major kinds of genetic actions outlined in FIG. 1 (deleterious mutations with largely recessive effects or overdominant alleles). If the evidence from studies of heterosis suggested great importance of overdominance, this would imply that overdominance is also important in causing inbreeding depression within populations. However, one must understand the differences between the types and frequencies of genetic variants that may cause inbreeding depression and heterosis (summarized in TABLE 2 and in the main text).

The genetic variants involved in heterosis

Inbreeding depression requires genetic variation in a population, whereas heterosis between two populations (or between the F_1 individuals of genetically uniform strains) depends on genetic differences between them (TABLE 2). Heterosis will largely be due to mutations with small detrimental effects, whereas rare mutations with moderately large effects predominate in inbreeding depression. One reason for this difference is that mild detrimental mutations with almost intermediate dominance can contribute to heterosis (part **c** of the figure in BOX 2). Another reason is that, although detrimental mutations remain rare in large populations, small-effect mutations can reach high frequencies by genetic drift in small populations. Fixation of different mutations in different populations causes heterosis (FIG. 1). It also lowers the homozygosity difference between inbred and outbred individuals in a population, therefore decreasing inbreeding depression (which will be caused by the remaining rare mutations with larger effects[112,113]).

In contrast, balancing selection maintains similar allele frequencies in different populations, unless selection differs between them[114]. In principle, alleles with heterozygote advantage at intermediate frequencies in populations could be the sole cause of inbreeding depression (although this is implausible given the evidence that most populations have many rare deleterious mutations and that balancing selection rarely maintains variants for a long period of time (BOX 4)). Heterozygote advantage is, however, much less likely to contribute to heterosis — two alleles from different populations or inbred strains might sometimes be overdominant by chance, but this is unlikely.

Hybrid breakdown between differently adapted populations

Determining the genetics underlying heterosis between populations is complicated by simultaneous hybrid breakdown, which can occur if the species (or lineages in inbreeding species) have accumulated adaptive differences. Differences selected in one species or the other (or both[99]) can cause Dobzhansky–Muller incompatibility[115] when homozygotes for recessive 'incompatible' multi-locus combinations are generated in the F_2 hybrid generation or in the F_1 generation when the interacting alleles have dominant effects (for example, in some cotton hybrids[116]). Such interactions are a form of outbreeding depression. Recessive genetic incompatibilities have been found between natural populations of two highly inbreeding species, *Arabidopsis thaliana*[117] and *Caenorhabditis elegans*[118].

Outbreeding depression
Reduced fitness of F_1 or F_2 individuals after a cross between two species or strains.

Genetic variance
The variance of trait values that can be ascribed to genetic differences among individuals. The total genetic variance in a trait can be dissected into additive, dominance and other components; in populations, these components depend on the frequencies of the alleles at loci affecting the trait.

Is continued heterosis in maize evidence for overdominant alleles? Decades of selective breeding in maize might have been expected to eliminate (purge) mutant alleles that lower yield, and it is often argued that the observed continued heterosis for yield implies an important role for overdominance in heterosis. However, this evidence is not definitive support for overdominant alleles. First, F_1 yield has been selected for in maize[36], so continued heterosis is not surprising. Second, purging during selection for yield may not entirely eliminate heterosis due to deleterious mutations. The improved inbred line performance could be due to the selection of alleles at loci that do not contribute to heterosis, whereas the heterosis could be caused by numerous small-effect mutations that are not purged by the selection to improve the line. We have just described evidence that deleterious mutations are prevalent in populations, so it is plausible that they should be present in maize strains. We also explained that genetic models of purging with inbreeding show that mildly detrimental alleles are eliminated only over hundreds or thousands of generations[32,33]. Although these models have not (to our knowledge) included artificial selection for characters such as yield, mutational load can probably persist even in highly homozygous strains under strong selection against low-yielding genotypes.

Can rare deleterious mutations alone account for most inbreeding depression? The results discussed so far show that some inbreeding depression is due to individually rare large-effect viability and fertility mutations. Of the remaining, often substantial, inbreeding depression, how much is contributed by rare, mildly detrimental mutations at many loci, versus loci with intermediate frequency alleles maintained by overdominant or other forms of balancing selection? Plausible values for spontaneous deleterious mutation rates and their effects (in the few model species in which they can be estimated (BOX 4)) can account for much of the observed inbreeding decline and genetic variance in fitness without invoking overdominant loci[37].

The biometric dissection of genetic variance into components of additive variance and dominance variance[38] might be expected to allow more direct tests of whether mutational load or heterozygote advantage contributes most to genetic variation in fitness. Loci with a heterozygote advantage cause high dominance genetic variance in populations[39], whereas the mutational model (FIG. 1) predicts that most genetic variation will be additive when the mutations are incompletely recessive[40,41]. Many characters related to fitness give results that are consistent with the mutational model[42] although, interestingly, some fitness-related characters — such as early female fecundity, viability and male mating success in *D. melanogaster*[42–45] and flower size and pollen viability in *M. guttatus*[46,47] — have too much additive genetic variation to be explained by deleterious mutation alone. However, this does not suggest a major role of heterozygote advantage because, as explained above, such loci would cause high dominance, rather than additive, genetic variance. These intriguing results suggest that some of the inbreeding depression for these traits is caused by alleles that are present at intermediate frequencies, perhaps through the action of some other form of balancing selection (BOX 2).

Genetic mapping studies

Determining the effects of genes that cause heterosis and inbreeding depression. Many of the studies discussed above infer the genetic basis of inbreeding depression indirectly or as average properties of the genes involved. Now that genetic markers can be developed in almost any organism of interest, modern genome-wide mapping approaches — such as quantitative trait locus mapping (QTL mapping) — could potentially identify individual loci that affect traits related to fitness and agronomic value, and could directly show the relative contributions of detrimental mutations and overdominant loci.

Box 4 | **Assessing the action of selection using DNA sequences**

Evidence for deleterious mutation using DNA sequence divergence and diversity

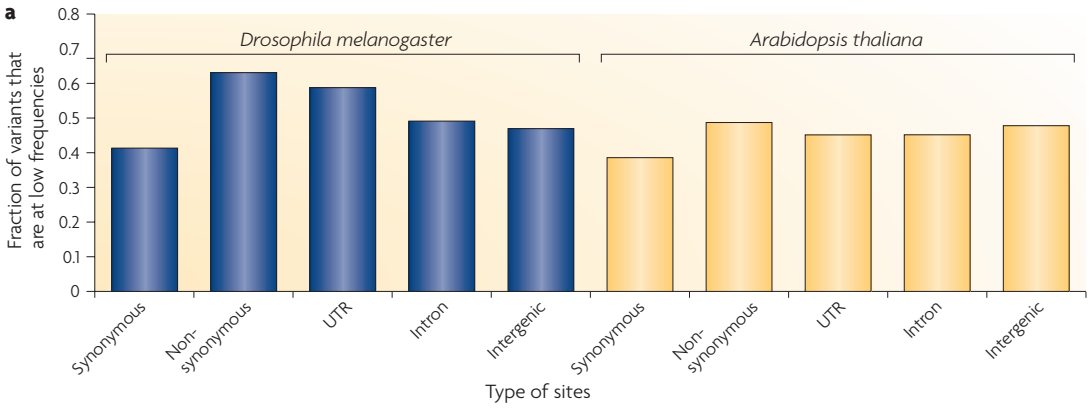
Comparisons of coding sequences of genes between different species show many fewer non-synonymous substitutions per site (d_n estimates) than synonymous ones (d_s values). Synonymous mutations are probably more often neutral with respect to fitness or impair fitness only slightly. Therefore $d_n < d_s$ implies that many non-synonymous mutations are deleterious[119,120]. Using the deficit of such substitutions to estimate the genomic deleterious mutation rate[121,122], current estimates are close to those obtained by directly ascertaining mutations in sampled genome regions in inbred mutation accumulation lines[123]. These inferences underestimate genomic deleterious mutation rates because they assume that selective constraints act only on non-synonymous mutations in coding regions, whereas selection also acts against variants in several types of non-coding regions [124–129].

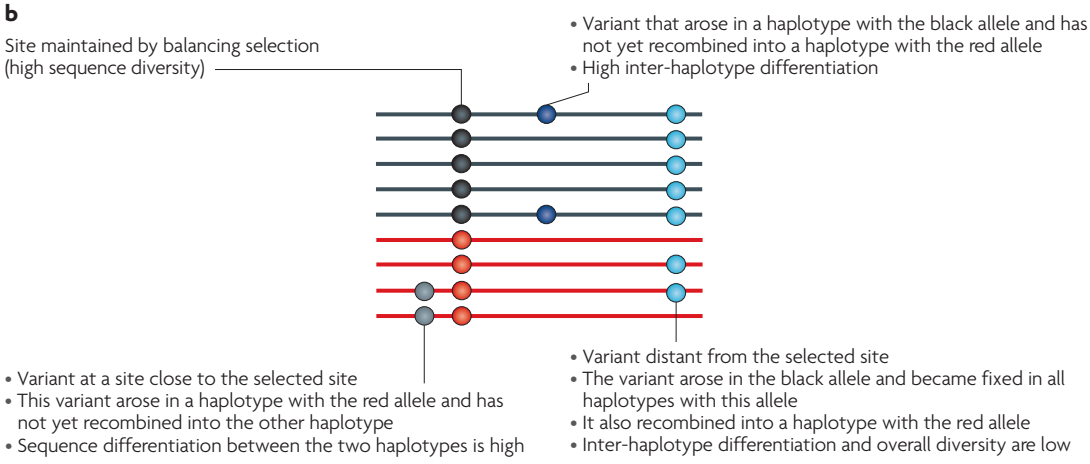
DNA sequence variants segregating in human and other populations also betray the action of selection. In the figure, part **a** shows data from *Drosophila melanogaster* and *Arabidopsis thaliana*. If synonymous variants are the least deleterious, the deficit of common variants at other types of sites implies selection against variants in these sequences. Combining such data with divergence from suitable related species, the strength of selection on mutations segregating in human populations is estimated to average a few per cent, with a wide distribution around this value[130]. The analyses suggest a tail of mutations with effects that are too weak to be detected in mutation accumulation experiments[131,132], and similar conclusions have been obtained in *D. melanogaster*[133–135].

Tests for balancing selection using DNA sequence diversity

Few cases of overdominance at single loci are well documented. Because alleles with heterozygote advantage can be maintained for long periods of time, their selection might be detectable because their sequences would have high nucleotide diversity relative to other genes in the same population. Part **b** shows the effect of long-term balancing selection on sequence variability in nearby regions of a sequence. Different alleles (indicated by red and black circles) each accumulate 'private variants'. Synonymous and non-coding variants in a region close to the site(s) under balancing selection (grey and red circles) will therefore have high diversity, but recombination breaks down the associations with distant regions (light blue circles). The 'migration' of variants through recombination into alleles other than those in which they arose (dark blue circles) is slow at the physical scale of a single locus[88].

Few such loci are so far known[136–139]. However, using high DNA sequence diversity to test for balancing selection results in an underestimation of its prevalence, because this method only detects alleles that are maintained for long periods of time and because the associations caused by mutations are restricted to genome regions very closely linked to variants that are the targets of the selection. Although such tests detect all forms of balancing selection, only some of these forms cause inbreeding depression (BOX 2). Part **a** is reproduced, with permission, from REF. 129 © (2008) Annual Reviews.





Additive variance
The component of genetic variance that is due to the additive effects of alleles. It is the primary contributor to resemblances between parents and offspring and to evolutionary responses to selection.

Dominance variance
The component of genetic variance that arises from deviations of heterozygotes from the mean of the two homozygotes (this will be large for loci with overdominant alleles).

Quantitative trait locus mapping
The use of genetic mapping to locate genome regions that contain a gene or genes that affect character values.

Mutation accumulation lines
Lines developed by multiple generations of breeding in such a way as to minimize the action of natural selection (for example, by using the same number of progeny from each breeding individual in each generation).

QTL mapping is, however, better suited to investigating heterosis in specific crosses than to studying inbreeding depression in a genetically diverse wild population. This is because, in wild populations, different genotypes tend to carry different alleles that lower the fitnesses of homozygotes (see above and BOX 2), which causes great variation in inbreeding depression from one genotype to another[48]. To adequately sample this diversity, experiments involving crosses between multiple parental genotypes are needed, which is currently impossible given the large effort and expense involved in studying even a single cross. Most QTL-mapping studies have therefore focused on heterosis in crop plants, which depends on inter-strain (or inter-species) differences (BOX 2) and can be studied using crosses between F_1 individuals.

Distinguishing overdominance from pseudo-overdominance in heterotic crosses. The first such study involved a cross between two maize inbred lines. Hundreds of F_3 progeny were backcrossed to both parents, and the progeny (grown in several locations) were measured for yield and other related traits and genotyped for markers[49]. All but one of the more than ten yield QTLs exhibited overdominance, and individual plant yields correlated strongly with the proportion of heterozygous markers in their genomes. A recent reanalysis that used more sophisticated methods resulted in more QTLs but again found pervasive overdominance[50]. Although these results might seem to provide strong evidence for overdominance, pseudo-overdominance (BOX 2) is not excluded. Indeed, when the genome region containing the QTL with the largest effect identified in the first study was fine mapped, pseudo-overdominance was confirmed; this overdominant QTL was found to consist of at least two linked smaller effect QTLs with deleterious recessive alleles in repulsion[51].

The limited resolution of most QTL studies (which rarely have the power to detect loci with small phenotypic effects, such as mildly deleterious mutations) means that linked genes with individually mild effects might often appear as a single major QTL, particularly in chromosomal regions with high gene density relative to recombination. This was confirmed by simulating numerous small-effect mutations distributed randomly among genes, taking into account the known patterns of recombination rates in the *D. melanogaster* genome[52,53]. As predicted, when the fitness effects were genetically mapped, most mutant loci were not individually detectable; instead large QTLs, consisting of linked mutations, were often detected, particularly in low recombination regions[53]. Indeed, when recombinant inbred lines are made in maize, the regions near the centromeres of all chromosomes become homozygous more slowly than regions with higher recombination, suggesting that these regions contain considerable recessive genetic load[54].

QTL-mapping studies in *Arabidopsis thaliana*[55], rapeseed[56] and other plant species have sometimes detected apparently overdominant QTLs that contribute to heterosis, but only rarely has pseudo-overdominance been definitively excluded. Breeding for several generations, to allow recombination to break down associations between deleterious mutations at different loci, has repeatedly revealed pseudo-overdominance[57]; a notable exception is the apparently overdominant locus in *A. thaliana*[58]. None of these cases has been re-examined or tested further with genetic markers or by fine-scale mapping. Even in cases in which alleles with apparent overdominance arose recently by mutation in a genetically homogeneous strain, pseudo-overdominance can be shown to have developed in the genome region involved[59,60].

Involvement of epistasis in heterosis in rice and A. thaliana. Another difficulty arises if epistatic interactions between genes are important (BOXES 2,3). Analyses of an early QTL-mapping experiment in a cross between the rice subspecies *indica* and *japonica*[61] suggest that pervasive epistasis between QTLs was important for heterosis[50]. Several other rice QTL-mapping experiments using the same rice subspecies have also detected substantial epistasis and apparent overdominance rather than true dominance[62–64], as have studies of *A. thaliana* crosses[65–67]. However, these QTL-mapping experiments on heterosis may not necessarily illuminate the genetics of inbreeding depression in natural populations. They involve crosses between divergent subspecies or, in *A. thaliana*, lines from different geographic regions. In such highly inbreeding plants, heterozygote advantage at any gene seems unlikely (BOX 3), but such 'wide crosses' often exhibit F_1 heterosis. However, subsequent generations may often have lower fitness owing to harmful epistatic interactions between alleles derived from their two parents — that is, 'hybrid breakdown'. Such 'incompatibilities' can also reduce heterosis in the F_1 generation and complicate its genetic analysis (BOX 3).

Mapping individual regions that cause heterosis using genome introgressions. To avoid epistatic interactions and the difficulties they pose for quantitative genetic analyses, multiple introgression lines were developed, each carrying a single small genome region from *Solanum pennellii* in an otherwise homozygous *Solanum lycopersicum* genetic background[68]. The lines were then each crossed to the inbred *S. lycopersicum* parent to test the different heterozygous segments for heterosis. These plants were monitored for 35 characters, along with the inbred parent and the homozygous introgression lines. For reproductive characters, the introgressed *S. pennellii* regions mostly reduced the values, acting like recessive deleterious alleles (not surprisingly, as the two species do not show overall F_1 heterosis[69]), but some regions showed heterozygote superiority.

Because the introgressed regions are large[69,70], pseudo-overdominance of the regions might explain the apparently overdominant regions; however, the authors argue against pseudo-overdominance, because only 0.7% of regions (that is, QTLs) for non-reproductive characters show apparent overdominance versus 15% of reproductive QTLs[68]. If deleterious alleles in repulsion phase in any region affect the two kinds of characters equally often, there should be no such difference. However, reproductive traits are the outcome of the entire developmental process and may be influenced by more genes than other traits — that is, they are more

Repulsion
The situation in a diploid organism when an allele of interest at one locus (for example, a mutant allele) came from a gamete contributed by one parent, and an allele at another locus came from the other parent (for example, the genotype +–/–+, in which – denotes mutant alleles and + denotes wild-type alleles).

Recombinant inbred lines
A population of fully homozygous individuals obtained through the repeated selfing of an F_1 hybrid.

Epistasis
The dependency of the effects of alleles at one locus on the genotypes at other loci in the genome.

Introgression
Crossing strains or species in such a way as to introduce some of the genome of one of the parents into that of the other, often by repeated backcrossing and selecting for certain genetic markers or phenotypic characters.

likely, on average, to be affected by several linked genes and therefore to show pseudo-overdominance. The average proportion of introgressions that significantly affect a reproductive trait was indeed about twice that for individual non-reproductive traits, which suggests that the chance of more than one gene in a region affecting a trait is higher for reproductive traits. This experiment therefore gives uncertain evidence for true overdominance as the main contributor to heterosis, and the problems of inferences from wide crosses outlined above[69] lessen its relevance for the genetics of inbreeding depression.

QTL analyses of inbreeding depression. As explained above, no study has attempted to sample the natural diversity in populations. A mapping study of embryonic survival and early growth and survival studied selfed progeny from a single loblolly pine[71,72]. Of the 19 QTLs detected, 16 QTLs affected embryonic survival, and these were all consistent with deleterious recessive mutations, whereas the 2 QTLs that affected height growth showed large effects with overdominant gene action (or pseudo-overdominance). To characterize the genetic basis of inbreeding depression, this sort of experiment should be extended to multiple individuals sampled from populations. New DNA sequencing technologies may greatly lower the costs and time required to genotype mapping populations[73] and might make population-scale QTL studies of inbreeding depression feasible. If inbreeding depression is mainly caused by rare mutations (as the numerous quantitative genetic studies in *D. melanogaster*, *M. guttatus* and other species suggest), different families should carry different QTLs. However, as explained above, the low resolution of QTL-mapping experiments limits the ability to discriminate the individual genes.

Epigenetics and differential gene expression

Many experiments have been done to test whether heterotic F_1 hybrids exhibit unusual patterns of gene expression or epigenetic DNA modification compared with their inbred parents. This approach is suggested by the observation that gene expression differences are common between inbred strains of a range of organisms that show hybrid vigour (reviewed in REF. 74), including wheat[75]. In seedlings of two maize inbred lines and their F_1 heterotic hybrid, for example, about 10% of almost 14,000 genes studied had significant expression differences[76]. In 78% of these, the expression in the F_1 hybrid was statistically indistinguishable from the mean expression value of the two parents (that is, the expression of the alleles at each locus show additivity), but some differentially expressed genes had expression levels in the F_1 hybrid that were higher or lower than one parent, and sometimes expression levels were higher or lower than both parents. Other studies report more non-additively expressed genes, including many with F_1 expression levels outside the parental range[77,78].

In principle, any of these patterns could be caused by deleterious mutations (FIG. 2) and could contribute to inbreeding depression and heterosis, because gene expression levels will not generally be linearly related to trait values. A single copy of a wild-type allele for an enzyme, in heterozygotes for loss-of-function mutations, often provides reaction rates close to those of wild-type homozygotes (BOX 2).

It has been suggested that the occurrence of insertion/deletion (indel) variants in 5′ regions between the alleles of genes that are differentially expressed in different rice strains may imply that expression differences are important for heterosis in these strains[5] (although this need not imply true overdominance, see FIG. 2b). However, there is no direct evidence that gene-expression differences in any of the genes studied actually contribute to heterosis. The few studies of whether the extent of heterosis in crosses correlates with the extent of differential gene expression have yielded inconsistent conclusions. Using 16 maize hybrids and their inbred parents, hybrid yield was found to correlate positively with the fraction of differentially expressed genes in immature ear tissue (which may simply reflect different degrees of relatedness) and with the fraction of genes with additive expression patterns[79]. In a study of 6 hybrids, the number of differentially expressed genes indeed correlated strongly with estimated sequence divergence between the parents, although only 25% of the differentially expressed genes were non-additively expressed in the hybrids[80]. In a larger sample of 25 hybrids, however, sequence divergence between parental lines correlated only weakly with the heterosis of several traits, so this study does not support a strong correlation between the fraction of differentially expressed genes and heterosis. Furthermore, differences in protein sequences were not excluded, and such polymorphisms are abundant in natural populations of many organisms, and could contribute to heterosis (BOX 4).

The correct control of gene expression is often important for fitness and yield and may contribute to inbreeding depression[81] (FIG. 2), but there is no evident reason why non-optimal gene expression should specifically result from inbreeding. Correct gene expression might be maintained by epigenetic 'crosstalk' between different alleles, so prolonged homozygosity might partially silence genes and normal expression might be restored in heterozygous hybrids. If this is the case, haploids that are derived from hybrids should perform better than haploids derived from inbreds; however, this was not found in maize[77]. Direct studies of DNA methylation in maize inbred lines and their hybrids have also failed to find spontaneous changes in methylation state in relation to heterozygosity[82].

Conclusions and remaining challenges

We have argued that the data on inbreeding depression, taken as a whole, are readily interpreted as the effects of homozygosity for deleterious mutations with largely recessive effects. The evidence suggests that the high fitness of heterozygotes compared with homozygotes rarely derives from individual overdominant loci, and only seems to do so because single-gene resolution is rarely possible in QTL mapping (and has not yet been achieved for such multi-locus situations as inbreeding depression). The main advance since Darwin's time is that the work of the early maize geneticists (whose hypotheses are summarized in FIG. 1) showed that inbreeding depression and

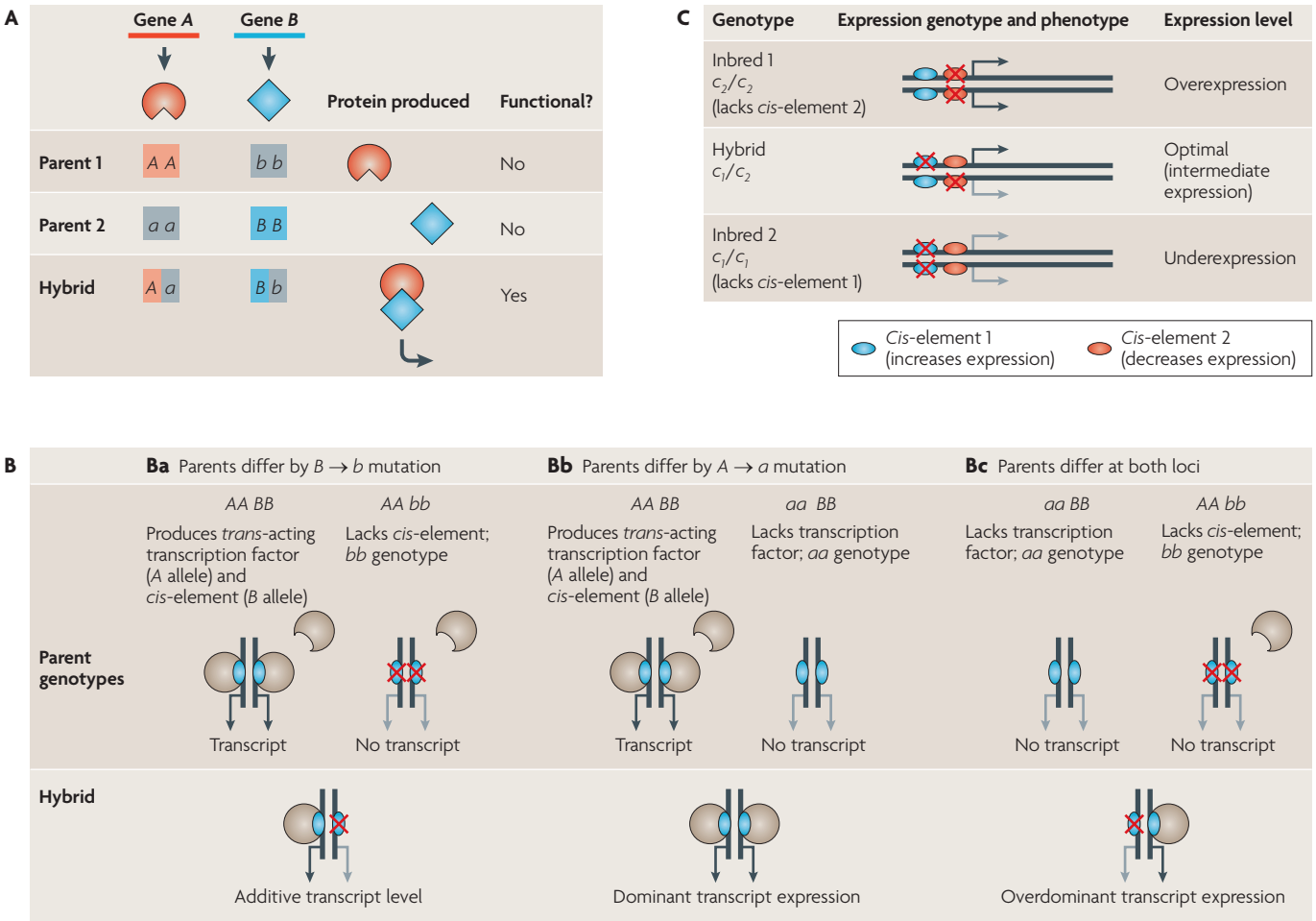


Figure 2 | **Molecular hypotheses for heterosis or heterozygote functional superiority.** Each of the three mechanisms shown is a special case of the types of genetic effects outlined in FIG. 1. Parts **A** and **B** show two-locus situations involving complementary interactions between non-allelic deleterious recessive mutations, whereas part **C** shows a single-locus situation that also involves pseudo-overdominance. **A** | Protein complexes, including *trans*-acting transcription factors, can yield apparent overdominance. Genes *A* and *B* each encode a component of such a complex. Each parent has a mutation and lacks one component, whereas their F_1 progeny receive functional alleles for both components and therefore have a functional complex (and higher fitness). **Ba** | Mutations (red crosses) in *cis*-regulatory regions (blue ovals) result in additive variation. Light grey arrows indicate that no transcript is produced, and dark grey arrows indicate high expression. **Bb** | Mutations in *trans*-acting transcription factors (beige circles) usually result in dominance of expression. **Bc** | Mutations in *cis*-regulatory regions and *trans*-acting transcription factors in two parent genotypes can yield (apparent) overdominance in expression levels[5]; neither parent has normal expression, but F_1 expression is high. **C** | Single-locus overdominance when optimal expression is between that of the two parents[74]. This might happen if one parent (inbred 1) overexpresses the gene owing to the lack of an expression-decreasing *cis*-regulatory element (*cis*-element 2; orange oval) and the other parent (inbred 2) has an allele that causes suboptimal expression owing to the lack of an expression-increasing *cis*-regulatory element (*cis*-element 1; blue oval). In the F_1 hybrid, the two mutations complement one another, to give a form of pseudo-overdominance. Part **b** is modified, with permission, from REF. 5 © (2008) Oxford University Press.

heterosis are genetic phenomena, as Darwin suspected. Much of the continued interest in inbreeding depression derives from the challenges in elucidating the action of the genes responsible and (as we have discussed) devising tests to determine whether overdominant genes add substantially to the contributions of deleterious mutations.

Given that individual overdominant genes have not been identified, the role of these genes remains unresolved. If the main contribution to inbreeding depression and heterosis comes from many rare, harmful, small-effect mutations that segregate at low frequencies, identifying individual loci may be impossible. Vast numbers of small-effect mutations probably exist at low frequencies in humans and underlie many common diseases, such as asthma (which explains why the genes that have been mapped in powerful association mapping analyses explain little of the total genetic variance[83]). However, as explained in BOX 2, many deleterious mutations probably have almost intermediate dominance and may not contribute much to inbreeding depression. This gives hope that alleles with large enough effects for study by QTL analyses may be involved in inbreeding depression.

Selection coefficient
The strength of selection, measured as the difference in fitness from genotypes of interest (for instance, a homozygote for a lethal allele has a selection coefficient of 1 if the fitness of the wild-type homozygote is denoted by 1).

Synonymous changes
Mutations or substitutions in a coding sequence are synonymous if they do not change the amino acid sequence of the protein encoded (non-synonymous changes are ones that do change the amino acid sequence).

Sophisticated analyses of QTL-fine-mapping experiments, starting with experimentally tractable numbers of founding genotypes, may soon allow nearly single-gene resolution of QTLs, and they should have the power to detect mutations that have effects of just a few per cent and that are present in just one of the founders[84–86].

Molecular evolutionary evidence. Our understanding of the main types of genetic effects that contribute to inbreeding depression may also advance through molecular population genetic analyses of genetic variation in fitness. These studies have already established that deleterious mutations occur in coding and non-coding regions (BOX 4). This evidence is independent of that from the experiments reviewed earlier in this article and comes from a larger number of species.

The availability of DNA sequence data has led to the development of approaches to estimate the overall distributions of the important parameters rather than to identify individual loci that contribute to inbreeding depression. Sophisticated molecular population genetic analyses are being developed that can integrate data on polymorphisms in species with information about sequence divergence between species and estimate mutation rates of deleterious amino acid variants and of deleterious mutations in non-coding regions; these methods are leading to a better understanding of the distribution of selection coefficients. BOX 4 also outlines how it is becoming possible to screen for alleles that are maintained by balancing selection. These studies have so far found little support for variants that are maintained in many genome regions by balancing selection, but finer-scale screens may alter this conclusion.

Many people would like to know the molecular basis for heterosis, given the striking benefits of crosses between inbred strains. However, there is probably no unitary molecular basis. Like other quantitative characters with multi-locus underlying genetics, fitness and yield characters probably do not share any common underlying properties, such as being controlled by differences in gene expression levels rather than protein sequences. Indeed, all of the major types of genetic action that can cause inbreeding depression and heterosis, as outlined in FIG. 1, can arise by various different molecular mechanisms, including detrimental mutations in coding sequences, or mutations that affect *cis*-regulatory regions, *trans*-acting transcription factors, or combinations of both (FIG. 2). Integrated approaches, which combine high-resolution QTL studies of trait variation with studies of gene expression at the population level, may show whether expression differences are important for variation in fitness. BOX 4 describes evidence for low-frequency, non-synonymous mutations in populations (which are probably deleterious mutations). Technical advances are making it possible to detect expression variants and to assess whether these are also rare[87]. If they are rare, low-expression alleles might often have their expression restored in heterozygotes by most other alleles from the population[81], which would argue for a contribution of such variants to genetic load.

Possible causes of overdominance. Finally, if overdominant genes contribute to inbreeding depression or fitness variation, it will be of interest for geneticists to understand the mechanisms that cause homozygosity for particular alleles to result in lower survival and reproductive capacities. Overdominance sometimes arises when alleles that are advantageous in heterozygotes spread or if the homozygotes (which are generated once the allele becomes common) suffer a disadvantage. If the homozygotes for a new allele confer a selective disadvantage, this will not prevent the spread of the allele to intermediate frequencies[88], as shown by the well-known example of the overdominant sickle-cell allele that protects against malaria[89]. Such events can be found in fine-scale genome screens, although the effects of recent changes in demographic history must be excluded because they can generate phenomena that are similar to those caused by selection. However, it seems unlikely that a sufficient number of such events are ongoing in most species to contribute greatly to inbreeding depression, particularly as such polymorphisms are often ephemeral and are usually replaced by others that confer the same benefit but without the disadvantage (such as the Duffy allele that also protects against malaria but does not carry the disadvantages of the sickle-cell allele[90]).

Nevertheless, the importance of overdominance relative to deleterious mutations cannot yet be quantified accurately. Surprisingly, their importance may soon be shown by molecular evolutionary approaches rather than by identifying individual loci through genome mapping.

1. Darwin, C. R. *The Effects of Cross and Self Fertilization in the Vegetable Kingdom* (John Murray, London, 1876).
2. Darwin, C. R. *The Various Contrivances by which Orchids are Fertilised by Insects*. (John Murray, London, 1862).
3. Darwin, C. R. *The Different Forms of Flowers on Plants of the Same Species* (John Murray, London, 1877).
4. McCune, A. R. *et al.* A low genomic number of recessive lethals in natural populations of bluefin killifish and zebrafish. *Science* **296**, 2398–2401 (2002).
5. Zhang, H.-Y. *et al.* A genome-wide transcription analysis reveals a close correlation of promoter INDEL polymorphism and heterotic gene expression in rice hybrids. *Mol. Plant* **1**, 720–731 (2008).
A fascinating, detailed study of gene expression differences in rice, together with information about DNA sequence differences in non-coding regions that are adjacent to genes. It also contains clear models that show the possible expression patterns that can arise.
6. Duvick, D. N. Biotechnology in the 1930s: the development of hybrid maize. *Nature Rev. Genet.* **2**, 69–74 (2000).
7. Grossniklaus, U., Nogler, G. A. & Dijk, P. J. v. How to avoid sex: the genetic control of gametophytic apomixis. *Plant Cell* **13**, 1491–1498 (2004).
8. Lewontin, R. C. *The Genetic Basis of Evolutionary Change* (Columbia Univ. Press, New York, 1974).
9. Crow, J. F. Mutation, mean fitness, and genetic load. *Oxf. Surv. Evol. Biol.* **9**, 3–42 (1993).
10. Barrière, A. *et al.* Detecting heterozygosity in shotgun genome assemblies: lessons from obligately outcrossing nematodes. *Genome Res.* **19**, 470–480 (2009).
11. Sved, J. A. An estimate of heterosis in *Drosophila melanogaster*. *Genet. Res.* **18**, 97–105 (1971).
12. Latter, B., Mulley, J., Reid, D. & Pascoe, L. Reduced genetic load revealed by slow inbreeding in *Drosophila melanogaster*. *Genetics* **139**, 287–297 (1998).
13. Willis, J. H. Genetic analysis of inbreeding depression caused by chlorophyll-deficient lethals in *Mimulus guttatus*. *Heredity* **69**, 562–572 (1992).
14. Klekowski, E. J., Lowenfeld, R. L. & Hepler, P. K. Mangrove genetics II. Outcrossing and lower spontaneous mutation rates in Puerto Rican Rhizophora. *Int. J. Plant Sci.* **155**, 373–381 (1994).
15. Ohnishi, O. Population genetics of cultivated buckwheat, *Fagopyrum esculentum* Moench. I. Frequency of chlorophyll-deficient mutants in Japanese populations. *Jpn J. Genet.* **57**, 623–639 (1982).
16. Ohnishi, O. Population genetics of cultivated buckwheat, *Fagopyrum esculentum* Moench. III. Frequency of sterility mutants in Japanese populations. *Jpn J. Genet.* **60**, 391–404 (1985).

17. Willis, J. H. The contribution of male sterility mutations to inbreeding depression in *Mimulus guttatus*. *Heredity* **83**, 337–346 (1999).
This genetic study extends the evidence for large-effect mutations that segregate in natural populations to species other than *D. melanogaster*.
18. Werren, J. in *The Natural History of Inbreeding and Outbreeding* (ed. Thornhill, N. W.) 42–59 (Univ. Chicago Press, 1993).
19. Henter, H. J. Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. *Evolution* **57**, 1793–1803 (2003).
20. Fisher, R. A. Average excess and average effect of a gene substitution. *Ann. Eugen.* **11**, 53–63 (1941).
An important theoretical paper that first introduced and showed the genetic transmission advantage of inbreeding.
21. Nagylaki, T. A model for the evolution of self fertilization and vegetative reproduction. *J. Theor. Biol.* **58**, 55–58 (1976).
22. Stebbins, G. L. *Variation and Evolution in Plants* (Columbia Univ. Press, New York, 1950).
23. Lloyd, D. G. Some reproductive factors affecting the selection of self-fertilization in plants. *Am. Nat.* **113**, 67–79 (1979).
24. Porcher, E. & Lande, R. The evolution of self-fertilization and inbreeding depression under pollen discounting and pollen limitation. *J. Evol. Biol.* **18**, 497–508 (2005).
An important, integrated model of outcrossing rate evolution that includes several biologically relevant processes.
25. Charlesworth, D. & Charlesworth, B. Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. *Evolution* **44**, 870–888 (1990).
26. Johnston, M. O. *et al.* Correlations among fertility components can maintain mixed mating in plants. *Am. Nat.* **173**, 1–11 (2009).
27. Byers, D. L. & Waller, D. M. Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *Annu. Rev. Ecol. Syst.* **30**, 479–513 (1999).
28. Carr, D. E. & Dudash, M. Recent approaches into the genetic basis of inbreeding depression in plants. *Philos. Trans. R. Soc. Lond. B* **358**, 1071–1084 (2003).
29. Crnokrak, P. & Barrett, S. C. D. Perspective: purging the genetic load: a review of the experimental evidence. *Evolution* **56**, 2347–2358 (2002).
30. Charlesworth, B., Charlesworth, D. & Morgan, M. T. Genetic loads and estimates of mutation rates in very inbred plant populations. *Nature* **347**, 380–382 (1990).
31. Ohta, T. & Cockerham, C. C. Detrimental genes with partial selfing and effects on a neutral locus. *Genet. Res.* **23**, 191–200 (1974).
32. Wang, J., Hill, W. G., Charlesworth, D. & Charlesworth, B. Dynamics of inbreeding depression due to deleterious mutations in small populations: I. Mutation parameters and inbreeding rate. *Genet. Res.* **74**, 165–178 (1999).
33. Charlesworth, D., Morgan, M. T. & Charlesworth, B. Inbreeding depression, genetic load and the evolution of outcrossing rates in a multi-locus system with no linkage. *Evolution* **44**, 1469–1489 (1990).
34. Willis, J. H. The role of genes of large effect on inbreeding depression in *Mimulus guttatus*. *Evolution* **53**, 1678–1691 (1999).
An ingenious experimental approach to understanding how much inbreeding depression can be accounted for by large-effect deleterious mutations.
35. Fox, C. W., Scheibly, K. L. & Reed, D. H. Experimental evolution of the genetic load and its implications for the genetic basis of inbreeding depression. *Evolution* **62**, 2236–2249 (2008).
36. Moll, R. H., Cock, C. C., Stuber, C. W. & Williams, W. P. Selection responses, genetic–environmental interactions, and heterosis with recurrent selection for yield in maize. *Crop Sci.* **18**, 641–645 (1978).
37. Charlesworth, D. & Charlesworth, B. Inbreeding depression and its evolutionary consequences. *Ann. Rev. Ecol. Syst.* **18**, 237–268 (1987).
38. Falconer, D. S. & Mackay, T. F. C. *Introduction to Quantitative Genetics* (Longman, Harlow, 1996).
39. Haldane, J. B. S. Parental and fraternal correlations in fitness. *Ann. Eugen.* **14**, 288–292 (1949).
40. Houle, D., Hoffmaster, D. K., Assimacopoulos, S. & Charlesworth, B. The genomic rate of mutation for fitness in *Drosophila*. *Nature* **359**, 58–60 (1992).
41. Mukai, T., Cardellino, R. A., Watanabe, T. K. & Crow, J. F. The genetic variance for viability and its components in a local population of *Drosophila melanogaster*. *Genetics* **78**, 1195–1208 (1974).
42. Charlesworth, B., Miyo, T. & Borthwick, H. Selection responses of means and inbreeding depression for female fecundity in *Drosophila melanogaster* suggest contributions from intermediate-frequency alleles to quantitative trait variation. *Genet. Res.* **89**, 85–91 (2007).
43. Charlesworth, B. & Hughes, K. A. in *Evolutionary Genetics: From Molecules to Morphology* (eds Singh, R. S. & Krimbas, C. B.) 369–392 (Cambridge Univ. Press, 2000).
44. Charlesworth, B. & Hughes, K. A. Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence. *Proc. Natl Acad. Sci. USA* **93**, 6140–6145 (1996).
45. Charlesworth, B. & Charlesworth, D. The genetic basis of inbreeding depression. *Genet. Res.* **74**, 329–340 (1999).
46. Kelly, J. K. & Willis, J. H. Deleterious mutations and genetic variation for flower size in *Mimulus guttatus*. *Evolution* **55**, 937–942 (2001).
47. Kelly, J. K. Deleterious mutations and the genetic variance of male fitness components in *Mimulus guttatus*. *Genetics* **164**, 1071–1085 (2003).
An integrated analysis that uses quantitative genetic approaches to detect the effects of deleterious mutations on a fitness-related character.
48. Schultz, S. & Willis, J. H. Individual variation in inbreeding depression: the roles of inbreeding history and mutation. *Genetics* **141**, 1209–1223 (1995).
The authors extend models that are used to predict the overall average inbreeding depression to predict the distribution of effects.
49. Stuber, C. W., Lincoln, S. E., Wolff, D. W., Helentjaris, T. & Lander, E. S. Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* **132**, 823–839 (1992).
50. Garcia, A., Wang, S., Melchinger, A. E. & Zeng, Z. B. Quantitative trait loci mapping and the genetic basis of heterosis in maize and rice. *Genetics* **180**, 1707–1724 (2008).
51. Graham, G., Wolff, D. & Stuber, C. Characterization of a yield quantitative trait locus on chromosome five of maize by fine mapping. *Crop Sci.* **37**, 1601–1610 (1997).
52. Latter, B. Mutant alleles of small effect are primarily responsible for the loss of fitness with slow inbreeding in *Drosophila melanogaster*. *Genetics* **148**, 1143–1158 (1998).
53. Noor, M. A. F., Cunningham, A. & Larkin, J. Consequences of recombination rate variation on quantitative trait locus mapping studies: simulations based on the *Drosophila melanogaster* genome. *Genetics* **159**, 581–588 (2001).
54. McMullen, M. D. *et al.* Genetic properties of the maize nested association mapping population. *Science* **325**, 737–740 (2009).
55. Mitchell-Olds, T. Interval mapping of viability loci causing heterosis in *Arabidopsis*. *Genetics* **140**, 1105–1109 (1995).
56. Radoev, M., Becker, H. & Ecke, W. Genetic analysis of heterosis for yield and yield components in rapeseed (*Brassica napus* L.) by quantitative trait locus mapping. *Genetics* **179**, 1547–1558 (2008).
57. Wright, S. *Evolution and the Genetics of Populations* Vol. 3 (Univ. Chicago Press, 1977).
58. Redei, G. P. Single locus heterosis. *Z. Indukt. Abstamm. Vererbungsl.* **93**, 164–170 (1962).
59. Schuler, J. F. Natural mutations in inbred lines of maize and their heterotic effect. I. Comparison of parent, mutant and their F1 hybrid in a highly inbred background. *Genetics* **39**, 908–922 (1954).
60. Schuler, J. F. & Sprague, G. F. Natural mutations in inbred lines of maize and their heterotic effect. II. Comparison of mother line versus mutant when outcrossed to related inbreds. *Genetics* **41**, 281–291 (1955).
An important early test to distinguish between true overdominance and pseudo-overdominance.
61. Xiao, J., Li, J., Yuan, L. & Tanksley, S. Dominance is the major genetic-basis of heterosis in rice as revealed by QTL analysis using molecular markers. *Genetics* **140**, 745–754 (1995).
62. Li, Z., Pinson, S. R. M., Park, W. D., Patterson, A. H. & Stansel, J. W. Epistasis for three grain yield components in rice (*Oryza sativa* L.). *Genetics* **145**, 453–465 (1997).
63. Li, Z. *et al.* Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. *Genetics* **158**, 1737–1753 (2001).
64. Luo, X. *et al.* Additive and over-dominant effects resulting from epistatic loci are the primary genetic basis of heterosis in rice. *J. Integr. Plant Biol.* **51**, 393–408 (2009).
65. Kusterer, B. *et al.* Heterosis for biomass-related traits in *Arabidopsis* investigated by quantitative trait loci analysis of the triple testcross design with recombinant inbred lines. *Genetics* **177**, 1839–1850 (2007).
66. Kusterer, B. *et al.* Analysis of a triple testcross design with recombinant inbred lines reveals a significant role of epistasis in heterosis for biomass-related traits in *Arabidopsis*. *Genetics* **175**, 2009–2017 (2007).
67. Melchinger, A. E. *et al.* Genetic basis of heterosis for growth-related traits in *Arabidopsis* investigated by testcross progenies of near-isogenic lines reveals a significant role of epistasis. *Genetics* **177**, 1827–1837 (2007).
68. Semel, Y. *et al.* Overdominant quantitative trait loci for yield and fitness in tomato. *Proc. Natl Acad. Sci. USA* **103**, 12981–12986 (2006).
69. Nakazato, T., Bogonovich, M. & Moyle, L. C. Environmental factors predict adaptive phenotypic differentiation within and between two wild Andean tomatoes. *Evolution* **62**, 774–792 (2008).
70. Eshed, Y. & Zamir, D. An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. *Genetics* **141**, 1147–1162 (1995).
71. Remington, D. & O'Malley, D. Whole-genome characterization of embryonic stage inbreeding depression in a selfed loblolly pine family. *Genetics* **155**, 337–348 (2000).
72. Remington, D. & O'Malley, D. Evaluation of major genetic loci contributing to inbreeding depression for survival and early growth in a selfed family of *Pinus taeda*. *Evolution* **54**, 1580–1589 (2000).
73. Huang, X. *et al.* High-throughput genotyping by whole-genome resequencing. *Genome Res.* **19**, 1068–1076 (2009).
74. Springer, N. & Stupar, R. Allelic variation and heterosis in maize: how do two halves make more than a whole? *Genome Res.* **17**, 264–275 (2007).
75. Song, X., Ni, Z., Yao, Y., Zhang, Y. & Sun, Q. Identification of differentially expressed proteins between hybrid and parents in wheat (*Triticum aestivum* L.) seedling leaves. *Theor. Appl. Genet.* **118**, 213–225 (2009).
76. Swanson-Wagner, R. A. *et al.* All possible modes of gene action are observed in a global comparison of gene expression in a maize F1 hybrid and its inbred parents. *Proc. Natl Acad. Sci. USA* **103**, 6805–6810 (2006).
77. Auger, D. *et al.* Nonadditive gene expression in diploid and triploid hybrids of maize. *Genetics* **169**, 389–397 (2005).
78. Uzarowska, A. *et al.* Comparative expression profiling in meristems of inbred-hybrid triplets of maize based on morphological investigations of heterosis for plant height. *Plant Mol. Biol.* **63**, 21–34 (2007).
79. Guo, M. *et al.* Genome-wide transcript analysis of maize hybrids: allelic additive gene expression and yield heterosis. *Theor. Appl. Genet.* **113**, 831–845 (2006).
80. Stupar, R. M. *et al.* Gene expression analyses in maize inbreds and hybrids with varying levels of heterosis. *BMC Plant Biol.* **8**, 33 (2008).
81. Lemos, B., Araripe, L. O., Fontanillas, P. & Hartl, D. L. Dominance and the evolutionary accumulation of *cis*- and *trans*-effects on gene expression. *Proc. Natl Acad. Sci. USA* **105**, 14471–14476 (2008).
82. Zhao, X., Chai, Y. & Liu, B. Epigenetic inheritance and variation of DNA methylation level and pattern in maize intra-specific hybrids. *Plant Sci.* **172**, 930–938 (2007).
83. Frazer, K. A., Murray, S. S., Schork, N. J. & Topol, E. J. Human genetic variation and its contribution to complex traits. *Nature Rev. Genet.* **10**, 241–251 (2009).
84. Valdar, W., Flint, J. & Mott, R. Simulating the collaborative cross: power of quantitative trait loci detection and mapping resolution in large sets of recombinant inbred strains of mice. *Genetics* **172**, 1783–1797 (2006).
85. Valdar, W. *et al.* Genome-wide genetic association of complex traits in heterogeneous stock mice. *Nature Genet.* **38**, 879–887 (2006).
86. Macdonald, S. & Long, A. Joint estimates of quantitative trait locus effect and frequency using synthetic recombinant populations of *Drosophila melanogaster*. *Genetics* **176**, 1261–1281 (2007).

87. Gruber, J. D., Genissel, A., Macdonald, S. & Long, A. How repeatable are associations between polymorphisms in *achaete–scute* and bristle number variation in *Drosophila*? *Genetics* **175**, 1987–1997 (2007).
88. Charlesworth, D. Balancing selection and its effects on sequences in nearby genome regions. *PLoS Genet.* **2**, e64 (2006).
89. Currat, M. *et al.* Molecular analysis of the β-globin gene cluster in the Niokholo Mandenka population reveals a recent origin of the β^S Senegal mutation. *Am. J. Hum. Genet.* **70**, 207–223 (2002).
90. Hamblin, M. T. & Rienzo, A. D. Detection of the signature of natural selection in humans: evidence from the Duffy blood group locus. *Am. J. Hum. Genet.* **66**, 1669–1679 (2000).
91. Helgason, A., Pálsson, S., Guðbjartsson, D. F., Kristjánsson, þ. & Stefánsson, K. An association between the kinship and fertility of human couples. *Science* **319**, 813–816 (2008).
92. Bittles, A. H. & Neel, J. V. The costs of human inbreeding and their implications for variations at the DNA level. *Nature Genet.* **8**, 117–121 (1994).
93. Stoltenberg, C., Magnus, P., Skrondal, A. & Lie, R. Consanguinity and recurrence risk of stillbirth and infant death. *Am. J. Public Health* **89**, 517–523 (1999).
94. Stoltenberg, C., Magnus, P., Skrondal, A. & Lie, R. Consanguinity and recurrence risk of birth defects: a population-based study. *Am. J. Med. Genet.* **82**, 423–428 (1999).
95. Rudan, I. *et al.* Inbreeding and risk of late onset complex disease. *J. Med. Genet.* **40**, 925–932 (2003).
96. Weeks, S. C., Reed, S., Ott, D. & Scanabissi, F. Inbreeding effects on sperm production in clam shrimp (*Eulimnadia texana*). *Evol. Ecol. Res.* **11**, 125–134 (2009).
97. Hoare, K. & Hughes, R. N. Inbreeding and hermaphroditism in the sessile, brooding bryozoan *Celleporella hyalina*. *Mar. Biol.* **139**, 147–162 (2001).
98. Husband, B. C. & Schemske, D. W. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**, 54–70 (1995).
99. Escobar, J., Nicot, A. & David, P. The different sources of variation in inbreeding depression, heterosis and outbreeding depression in a metapopulation of *Physa acuta*. *Genetics* **180**, 1593–1608 (2008).
100. Dolgin, E., Charlesworth, B., Baird, S. & Cutter, A. Inbreeding and outbreeding depression in *Caenorhabditis* nematodes. *Evolution* **61**, 1339–1352 (2007).
101. Weller, S. G., Sakai, A. K., Thai, D. A., Tom, J. & Rankin, A. E. Inbreeding depression and heterosis in populations of *Schiedea viscosa*, a highly selfing species. *J. Evol. Biol.* **18**, 1434–1444 (2005).
102. Richards, C. Inbreeding depression and genetic rescue in a plant metapopulation *Am. Nat.* **155**, 383–394 (2000).
103. Crow, J. F. & Simmons, M. J. in *The Genetics and Biology of Drosophila* (eds Ashburner, M., Carson, H. L. & Thompson, J. N.) 1–35 (Academic Press, London, 1983).
104. Hoffmann, A. A. & Rieseberg, L. H. Revisiting the impact of inversions in evolution: from population genetic markers to drivers of adaptive shifts and speciation. *Ann. Rev. Ecol. Evol. Syst.* **39**, 21–42 (2008).
105. Dyer, K. A., Charlesworth, B. & Jaenike, J. Chromosome-wide linkage disequilibrium as a consequence of meiotic drive *Proc. Natl Acad. Sci. USA* **104**, 1587–1592 (2007).
106. Glemin, S., Bataillon, T., Ronfort, J., Mignot, A. & Olivieri, I. Inbreeding depression in small populations of self-incompatible plants. *Genetics* **159**, 1217–1229 (2001).
107. Pankey, M. & Wares, J. Overdominant maintenance of diversity in the sea star *Pisaster ochraceus*. *J. Evol. Biol.* **22**, 80–87 (2009).
108. Scoville, A., Lee, Y. W., Willis, J. H. & Kelly, J. K. The contribution of chromosomal polymorphisms to the G-matrix of *Mimulus guttatus*. *New Phytol.* **183**, 803–815 (2009).
109. Fishman, L. & Saunders, A. Centromere-associated female meiotic drive entails male fitness costs in monkeyflowers. *Science* **322**, 1559–1562 (2008).
110. Williams, W. Heterosis and the genetics of complex characters. *Nature* **184**, 527–530 (1959).
111. Schnell, F. & Cockerham, C. Multiplicative vs. arbitrary gene action in heterosis. *Genetics* **131**, 461–469 (1992).
112. Bataillon, T. & Kirkpatrick, M. Inbreeding depression due to mildly deleterious mutations in finite populations: size does matter. *Genet. Res.* **75**, 75–81 (2000).
113. Glémin, S., Ronfort, J. & Bataillon, T. Patterns of inbreeding depression and architecture of the load in subdivided populations. *Genetics* **165**, 2193–2212 (2003).
By analysing a model of deleterious mutations in a biologically realistic model of population structure, the authors reveal heterosis in inter-population crosses and within-population inbreeding depression.
114. Schierup, M. H., Vekemans, X. & Charlesworth, D. The effect of subdivision on variation at multi-allelic loci under balancing selection. *Genet. Res.* **76**, 51–62 (2000).
115. Coyne, J. A. & Orr, H. A. *Speciation* (Sinauer, Sunderland, 2004).
116. Song, L., Guo, W. & Zhang, T. Interaction of novel Dobzhansky–Muller type genes for the induction of hybrid lethality between *Gossypium hirsutum* and *G. barbadense* cv. Coastland R4-4. *Theor. Appl. Genet.* **119**, 33–41 (2009).
117. Bomblies, K., Lempe, J., Dangl, J. & Weigel, D. Autoimmune response as a mechanism for a Dobzhansky–Muller-type incompatibility syndrome in plants. *PLoS Biol.* **5**, 1962–1972 (2007).
118. Seidel, H. S., Rockman, M. V. & Kruglyak, L. Widespread genetic incompatibility in *C. elegans* maintained by balancing selection. *Science* **319**, 589–594 (2008).
119. Hurst, L. D. Genetics and the understanding of selection. *Nature Rev. Genet.* **10**, 83–93 (2009).
120. Yang, J., Gu, Z. & Li, W. Rate of protein evolution versus fitness effect of gene deletion. *Mol. Biol. Evol.* **20**, 772–774 (2003).
121. Kondrashov, A. S. & Crow, J. F. A molecular approach to estimating the human deleterious mutation-rate. *Hum. Mutat.* **2**, 229–234 (1993).
122. Keightley, P. D. & Eyre-Walker, A. Deleterious mutations and the evolution of sex. *Science* **290**, 331–333 (2000).
123. Haag-Liautard, C. *et al.* Direct estimation of per nucleotide and genomic deleterious mutation rates in *Drosophila*. *Nature* **445**, 82–85 (2007).
This paper provides direct evidence that the deleterious mutation rate is high in *D. melanogaster*.
124. Haddrill, P. R., Charlesworth, B., Halligan, D. L. & Andolfatto, P. Patterns of intron sequence evolution in *Drosophila* are dependent upon length and GC content. *Genome Biol.* **6**, R67 (2005).
125. Katzman, S. *et al.* Human genome ultraconserved elements are ultraselected. *Science* **317**, 915 (2007).
126. Parmley, J. L., Chamary, J. V. & Hurst, L. D. Evidence for purifying selection against synonymous mutations in mammalian exonic splicing enhancers. *Mol. Biol. Evol.* **23**, 301–309 (2006).
127. Keightley, P. D., Kryukov, G. V., Sunyaev, S., Halligan, D. L. & Gaffney, D. J. Evolutionary constraints in conserved nongenic sequences of mammals. *Genome Res.* **15**, 1373–1378 (2006).
128. Asthana, S. *et al.* Widely distributed noncoding purifying selection in the human genome. *Proc. Natl Acad. Sci. USA* **104**, 12410–12415 (2007).
129. Wright, S. & Andolfatto, P. The impact of natural selection on the genome: emerging patterns in *Drosophila* and *Arabidopsis*. *Annu. Rev. Ecol. Evol. Syst.* **39**, 193–213 (2008).
130. Eyre-Walker, A., Woolfit, M. & Phelps, T. The distribution of fitness effects of new deleterious amino acid mutations in humans. *Genetics* **173**, 891–900 (2006).
The authors make sophisticated use of population genetics theory to estimate the distribution (rather than the average value) of selection coefficients of deleterious mutations.
131. Boyko, A. R. *et al.* Assessing the evolutionary impact of amino acid mutations in the human genome. *PLoS Genet.* **4**, e1000083 (2008).
132. Keightley, P. & Halligan, D. Analysis and implications of mutational variation. *Genetica* **136**, 359–369 (2009).
133. Loewe, L., Charlesworth, B., Bartolomé, C. & Nöel, V. Estimating selection on nonsynonymous mutations. *Genetics* **172**, 1079–1092 (2006).
134. Loewe, L. & Charlesworth, B. Inferring the distribution of mutational effects on fitness in *Drosophila*. *Biol. Lett.* **2**, 426–430 (2006).
135. Keightley, P. & Eyre-Walker, A. Joint inference of the distribution of fitness effects of deleterious mutations and population demography based on nucleotide polymorphism frequencies. *Genetics* **177**, 2251–2261 (2007).
136. Bubb, K. L. *et al.* Scan of human genome reveals no new loci under ancient balancing selection. *Genetics* **173**, 2165–2177 (2006).
137. Asthana, S., Schmidt, S. & Sunyaev, S. A limited role for balancing selection. *Trends Genet.* **21**, 30–32 (2005).
References 136 and 137 give evidence that overdominance is not common.
138. Fumagalli, M. *et al.* Widespread balancing selection and pathogen-driven selection at blood group antigen genes. *Genome Res.* **19**, 199–212 (2009).
139. Calafell, F. *et al.* Evolutionary dynamics of the human *ABO* gene. *Hum. Genet.* **124**, 123–135 (2008).
140. Moss, D., Arce, S., Otoshi, C. & Moss, S. Inbreeding effects on hatchery and growout performance of Pacific white shrimp, *Penaeus* (*Litopenaeus*) *vannamei*. *J. World Aquacult. Soc.* **39**, 467–476 (2008).
141. Richards, C. M., Church, S. & McCauley, D. E. The influence of population size and isolation on gene flow by pollen in *Silene alba*. *Evolution* **53**, 63–73 (1999).
142. Mori, K., Saito, Y., Sakagami, T. & Sahara, K. Inbreeding depression of female fecundity by genetic factors retained in natural populations of a male-haploid social mite (Acari: Tetranychidae). *Exp. Appl. Acarol.* **39**, 15–23 (2005).
143. Schneller, J. J. & Holderegger, R. Vigor and survival of inbred and outbred progeny of *Athyrium filix-femina*. *Int. J. Plant Sci.* **158**, 79–82 (1997).
144. Klekowski, E. J. Genetic load in *Osmunda regalis* populations. *Am. J. Bot.* **60**, 146–154 (1973).
The studies reported in references 143 and 144 show evidence for recessive large-effect deleterious mutations in natural populations of ferns, a type of organism that should be more widely used in such studies.
145. Keller, L. F. Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution* **52**, 240–250 (1998).
146. Ritland, K. Inferences about inbreeding depression based upon changes of the inbreeding coefficient. *Evolution* **44**, 1230–1241 (1990).
147. Liautard, C. & Sundstrom, L. Estimation of individual level of inbreeding using relatedness measures in haplodiploids. *Insectes Soc.* **52**, 323–326 (2005).
148. Camara, M., Evans, S. & Langdon, C. Parental relatedness and survival of Pacific oysters from a naturalized population. *J. Shellfish Res.* **27**, 323–336 (2008).
149. Herlihy, C. R. & Eckert, C. G. Genetic cost of reproductive assurance in a self-fertilizing plant. *Nature* **416**, 320–323 (2002).
150. Bierne, N., S. Launey, Y. Naciri-Graven & Bonhomme, F. Early effect of inbreeding as revealed by microsatellite analyses on *Ostrea edulis* larvae. *Genetics* **148**, 1893–1906 (2000).
151. Launey, S. & Hedgecock, D. High genetic load in the pacific oyster *Crassostrea gigas*. *Genetics* **159**, 255–265 (2001).
152. Fu, Y.-B. & Ritland, K. Evidence for the partial dominance of viability genes in *Mimulus guttatus*. *Genetics* **136**, 323–331 (1993).
153. Fu, Y.-B. & Ritland, K. On estimating the linkage of marker genes to viability genes controlling inbreeding depression. *Theor. Appl. Genet.* **88**, 925–932 (1994).
154. Haag, C. & Ebert, D. D. Genotypic selection in *Daphnia* populations consisting of inbred sibships. *J. Evol. Biol.* **20**, 881–891 (2007).

Acknowledgements
This research was supported by US National Institutes of Health grant GM073990 to J.H.W. We thank B. Charlesworth for discussions.

FURTHER INFORMATION
John H. Willis's homepage:
http://www.biology.duke.edu/willislab
***Nature Reviews Genetics* article series on Fundamental Concepts in Genetics:** http://www.nature.com/nrg/series/fundamental/index.html

ALL LINKS ARE ACTIVE IN THE ONLINE PDF