

# Survival of the steepest: hypersensitivity to mutations as an adaptation to soft selection

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robustness;  
soft selection.

## Abstract

Darwinian evolution favours genotypes with high fitness ('survival of the fittest'). Models of quasi-species evolution, however, suggest that in some cases selection may favour genotypes that are more robust against the impact of mutations ('survival of the flattest') even if these genotypes have lower fitness. I show that the opposite effect will be observed if competition occurs during development (e.g. among embryos or ovules) or before the adult phase (e.g. among the progeny of an individual). If viability is not affected by selection at these initial stages (soft selection), the genotypes that are more sensitive to the effects of mutations may increase in frequency because they get rid more easily of deleterious mutations. In a simple theoretical model of mutation and selection, genotypes located in steeper regions of the fitness surface are favoured ('survival of the steepest') even if they do not have higher viability, and even if they have slightly deleterious effects. Hypersensitive genes are potentially harmful for the individual, but with soft selection during the juvenile phase they persist in the genome because they reduce competition with their mutants. Soft selection occurs in practically all vascular plants and in many animals, therefore antirobustness may be a very common feature of the genome of multicellular organisms.

## Introduction

### Survival of the flattest: hard selection leads to robustness

Natural selection usually favours genotypes with higher reproductive rate ('survival of the fittest'). Quasi-species models (Eigen, 1971; Eigen & Schuster, 1979; Eigen *et al.*, 1989), however, suggest that selection, at least in an asexual population and at high mutation rates, should maximize the overall replication rate of a cloud of genotypes connected by mutation. Thus, a fast-replicating organism that occupies a high and narrow peak in a fitness landscape (that is, whose nearby mutants have a very low fitness) can be displaced by an organism that occupies a lower but flatter peak. In other words, selection may favour higher genetic

robustness in spite of lower fitness. A 'flatter' region of the fitness surface corresponds to a robust genotype, and quasi-species models suggest that 'survival of the flattest' may be as important as 'survival of the fittest', at least in microbes (Schuster & Swetina, 1988). 'Survival of the flattest' has been shown (Wilke *et al.*, 2001) in competition experiments between digital organisms: at high mutation rates, competition favoured the genotypes located in flatter regions of the fitness surface even though these genotypes occupied lower fitness peaks.

The concept of genetic robustness (de Visser *et al.*, 2003) is similar to 'canalization' (Waddington, 1959), the capacity of genotypes to minimize the effects of mutations. Genetic robustness can be, in principle, of two kinds. One, which we may call 'auto-robustness', is the capacity of a genetic element to minimize the effects of mutations on itself (e.g. incomplete dominance). The second type, which we may call 'modifier-robustness', is the capacity of a genetic element to induce robustness somewhere else in the genome (e.g. a gene for diploidy).

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In the case of auto-robustness, because the mutants of a robust genotype have high viability, they can provide back mutations with higher frequency, and in asexual populations robust genes can increase in frequency because of the mutational backflow they receive from their mutant neighbours (Hermisson *et al.*, 2002). Although back-mutation is not needed for modifier-type robustness (see, for example, Gardner & Kalinka, 2006), the strength of selection for mutation robustness is expected to be of the same order as the mutation rate. Therefore, a high mutation rate is essential for the evolution of both kinds of genetic robustness (Wagner *et al.*, 1997; de Visser *et al.*, 2003).

In sexual populations, the situation is more complex and the evolution of robustness may also be driven by recombination rather than solely by new mutations (Wagner *et al.*, 1997; de Visser *et al.*, 2003): recombination pulls apart coadapted gene complexes, leading to a recombination load, which might impose selection for certain types of genetic robustness that would alleviate this; furthermore, in the case of modifier-type robustness, recombination may break down linkage disequilibrium between the modifier for robustness and the deleterious mutations that accumulate at other loci because of the modifier effect, promoting the evolution of mutational robustness (Gardner & Kalinka, 2006).

### Survival of the steepest: soft selection leads to antirobustness

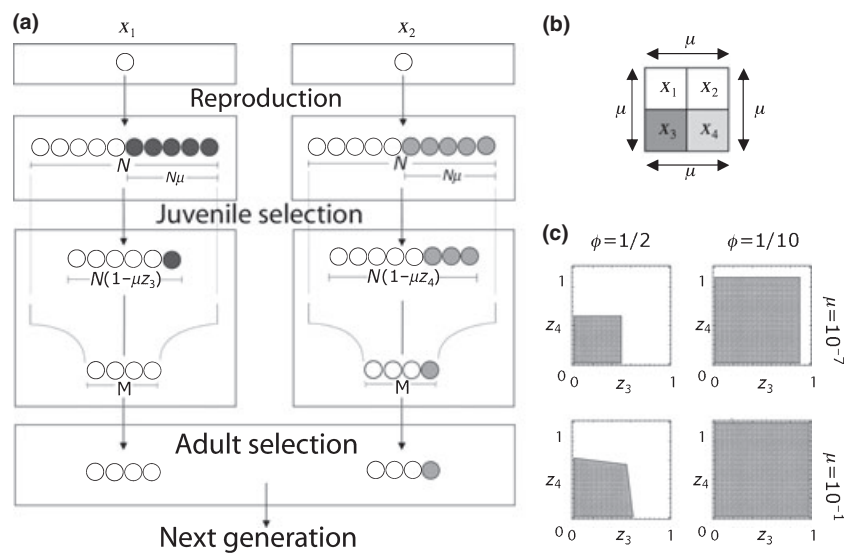
Evolution is usually (including the scenario described above) modelled with assumptions of 'hard' selection, i.e. selection among all the individuals of a population, in the adult phase before reproduction: each individual that dies decreases fitness. There are cases, however, in which it is not true that each death reduces fitness. The concept

is often called 'soft selection' (Buchholz, 1922; Wallace, 1981; Klekowski, 1988). Wallace (1981) defines soft selection in terms of 'unit biological spaces' in which one and only one individual can survive: if a space is occupied by more than one individual, only one (the fittest) will survive. Selection is 'soft' because one individual will always survive in one space. The viability of a genotype is scaled against its immediate competitors for that space, and not against the whole population.

For example, consider (Fig. 1a) an individual with  $N$  offspring. Only  $M$  of these  $N$  offspring can go on to the adult phase ( $M$  is the number of unit biological spaces) in any case (because of limited resources available from the parents, for example). If  $M < N$ , the juvenile individuals are in competition (only) with each other to go on to the adult phase. Discarding a small part of the progeny at the initial stage of competition among offspring will have no effect on viability as long as  $M < N$  and it may even be beneficial because it allows to get rid of deleterious mutants from the progeny.

The point of this paper is that with soft selection in the juvenile phase, the genotypes that are more sensitive to the effects of mutations ('hypersensitive' or 'antirobust') will get rid more easily of deleterious mutations and therefore will have an advantage in the adult phase when competing with other genotypes. This effect does not depend on the rate of back mutations and therefore is arguably of a higher magnitude than the effect leading to selection for robustness (Otto & Hastings, 1998). With soft selection, the selective coefficient against mutants can be much higher and lead to a bias in favour of antirobust genotypes even if they do not have higher viability, and possibly even if they have slightly lower viability. The crucial point is not simply soft selection, but multi-level selection during the life-cycle of an individual, with a soft selection sieve during the juvenile phase.

**Fig. 1** An example of soft selection (a). Wild types  $x_1$  and  $x_2$  do not differ in fitness but their mutants (b) do ( $x_1$  is antirobust with  $z_3 > z_4$ ). With soft selection,  $x_1$  may increase in frequency because its mutants are eliminated more easily from the population before the adult phase and therefore the progeny of  $x_1$  is more competitive than the progeny of  $x_2$  in the adult phase. Loss of viability does not occur in most cases (grey area in c).



The idea seems intuitive but there is a problem that requires a quantitative analysis. Making a mutation nastier would impact negatively on both the juvenile and the adult stage, if it is expressed in both stages. Will antirobustness evolve only for genes expressed in the juvenile phase? If the gene is only expressed in the juvenile phase, however, there seems to be no benefit of weeding it out. The solution to this question is that with juvenile soft selection deleterious mutations are wiped out before adult selection, but the extent to which this is true depends on how efficient the soft selection sieve is, which in turn depends, among other things, on the strength of selection itself. How strong should selection against deleterious mutations be? For what mutation rates is this possible? How different should selection be in the adult and juvenile phases?

These questions are analysed in the following section with a population genetics model of soft selection in the juvenile phase followed by selection in the adult phase. In the second part, I will show simulations of competition experiments of populations under a similar scenario. In the third part, I will discuss the relevance of soft selection for animal and plant life cycles, as well as similar concepts developed for microbes.

### The 4-allele model

#### The model

Consider a locus with four alleles  $x_1, x_2, x_3$  and  $x_4$ ;  $x_1$  and  $x_2$  are the wild type alleles and have the same effect and fitness;  $x_3$  and  $x_4$  are the mutants of, respectively,  $x_1$  and  $x_2$ , and can have different effects and fitness. Mutations occur at a rate  $\mu$  according to the scheme in Fig. 1b. Individuals with allele  $x_i$  have fitness  $1 - s_i$  in the adult phase and  $1 - z_i$  in the juvenile phase. If  $N$  and  $N'_i$  are the number of offspring of an individual with allele  $i$ , respectively before and after juvenile selection ( $N$  is the same for all alleles), then, assuming  $z_1 = z_2 = 0$ ,

$$N'_1 = N(1 - \mu z_3)$$

$$N'_2 = N(1 - \mu z_4)$$

$$N'_3 = N[1 - z_3 - \mu(z_4 - 2z_3)]$$

$$N'_4 = N[1 - z_4 - \mu(z_3 - 2z_4)]$$

$M (< N)$  is the maximum number of individuals that may go on to the adult phase. The parameter  $\phi = M/N$  can be viewed as the ‘buffering’ potential of the juvenile phase. If  $\phi = 1$  all individuals generated must reproduce, or a loss of viability occurs; if  $\phi \rightarrow 0$  then only an infinitesimal number of the  $N$  juvenile individuals will go on to the adult phase in any case, and discarding part of the progeny will never have any effect on viability. In general, the conditions for no loss of viability are given by  $N'_i \geq M$ . Unless  $M \approx N$  (no soft selection), loss of viability will not occur for most values of  $z_3$  and  $z_4$  (Fig. 1c). Even for a very modest level of soft selection ( $\phi = 1/2$ ), a model that does not include loss of viability is appropriate as long as we exclude from the analysis lethal and semi-lethal alleles, and for a not too extreme case of soft selection ( $\phi = 1/10$ ) even semi-lethal alleles are compatible with the model (Fig. 1c). Furthermore, with reasonable values of the mutation rates, loss of viability would affect significantly only the recurrence equations for the mutant alleles ( $x_3$  and  $x_4$ ), not the wild type alleles ( $x_1$  and  $x_2$ ), and therefore its effect is negligible even with lethal alleles and high values of  $\phi$ . In what follows, therefore, I will assume for simplicity no loss of viability.

The recurrence equations for this genetic system are (see Table 1):

$$Tx'_1 = x_1(1 - s_1)(1 - 2\mu)(1 - z_1)/\alpha + x_2(1 - s_2)\mu(1 - z_1)/\beta + x_3(1 - s_3)\mu(1 - z_1)/\chi$$

$$Tx'_2 = x_1(1 - s_1)\mu(1 - z_2)/\alpha + x_2(1 - s_2)(1 - 2\mu)(1 - z_2)/\beta + x_4(1 - s_4)\mu(1 - z_2)/\delta$$

$$Tx'_3 = x_1(1 - s_1)\mu(1 - z_3)/\alpha + x_3(1 - s_3)(1 - 2\mu)(1 - z_3)/\chi + x_4(1 - s_4)\mu(1 - z_3)/\delta$$

$$Tx'_4 = x_2(1 - s_2)\mu(1 - z_4)/\beta + x_3(1 - s_3)\mu(1 - z_4)/\chi + x_4(1 - s_4)(1 - 2\mu)(1 - z_4)/\delta$$

**Table 1** The offspring and fitness of the four alleles in the model.

		Offspring			
Fitness		$x_1$	$x_2$	$x_3$	$x_4$
$x_1$	$1 - s_1$	$(1 - 2\mu)(1 - z_1)/\alpha$	$\mu(1 - z_2)/\alpha$	$\mu(1 - z_3)/\alpha$	0
$x_2$	$1 - s_2$	$\mu(1 - z_1)/\beta$	$(1 - 2\mu)(1 - z_2)/\beta$	0	$\mu(1 - z_4)/\beta$
$x_3$	$1 - s_3$	$\mu(1 - z_1)/\chi$	0	$(1 - 2\mu)(1 - z_3)/\chi$	$\mu(1 - z_4)/\chi$
$x_4$	$1 - s_4$	0	$\mu(1 - z_2)/\delta$	$\mu(1 - z_3)/\delta$	$(1 - 2\mu)(1 - z_4)/\delta$

$$\alpha = 1 - (1 - 2\mu)z_1 - \mu z_2 - \mu z_3.$$

$$\beta = 1 - (1 - 2\mu)z_2 - \mu z_4 - \mu z_1.$$

$$\chi = 1 - (1 - 2\mu)z_3 - \mu z_4 - \mu z_1.$$

$$\delta = 1 - (1 - 2\mu)z_4 - \mu z_2 - \mu z_3.$$

where  $T$  is a normalizing factor obtained by summing the right-hand side of the four above equations;  $\alpha$ ,  $\beta$ ,  $\chi$  and  $\delta$  are normalizing factors (see Table 1) of the offspring frequencies: the offspring frequencies just after reproduction (the numerators in the equations above and in Table 1) are normalized (on the assumption of no loss of viability) after juvenile selection, by dividing them by the total frequencies of the offspring remained after juvenile selection ( $\alpha$ ,  $\beta$ ,  $\chi$  and  $\delta$  as appropriate, see Table 1). These normalized frequencies go on to the adult phase, where another round of (hard) selection occurs. With  $z_1 = 0$  juvenile selection is excluded from the model.

The equilibrium frequencies of the four alleles can be found by specifying the parameters  $\mu$ ,  $s_1$  and  $z_1$  for the system above and calculating the leading eigenvector.

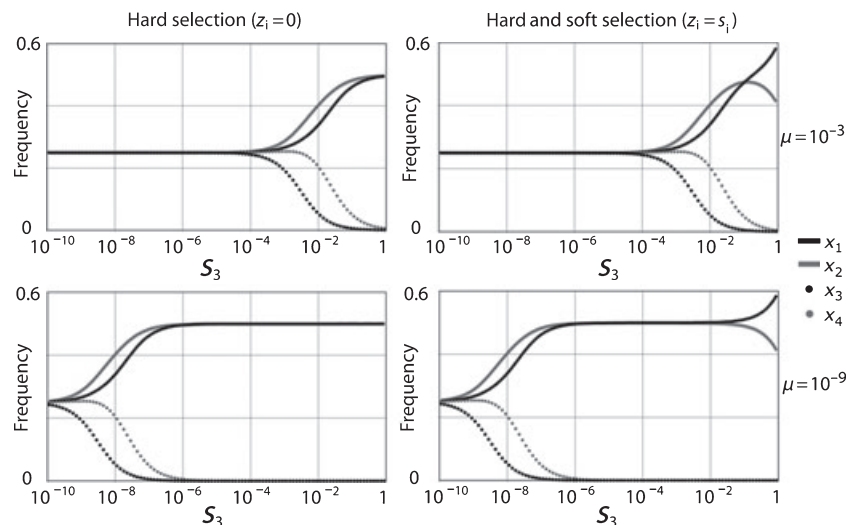
## Results

We can assume, for the moment, that  $s_1 = s_2 = z_1 = z_2 = 0$ . The situation in which allele  $x_1$  is less robust than  $x_2$ , is given by  $s_3 > s_4 > 0$  and  $z_3 > z_4 > 0$ . With no juvenile selection ( $z_3 = z_4 = 0$ ) the robust allele  $x_2$  increases in frequency as  $s_3$  increases, over a threshold approximately  $s_3 \approx \mu$ , irrespective of the value of  $\mu$ ; this means that when selection is too weak compared with the mutation rate, there is no appreciable bias because the differential survival of mutants, and therefore the differential amount of back mutations, is negligible. This effect is well known in the general theory of robustness and is captured by the left part ( $s_3 \ll \mu$ ) of each plot in Fig. 2 (moreover, even though in an infinite population, a bias can be produced with low selection coefficients, in a real population drift would prevail). For a bias in frequency to exist, however,  $s_3$  must also be not too high: if it is much higher than the mutation rate most of the mutants disappear and cannot contribute to back mutations at all. This effect is also well

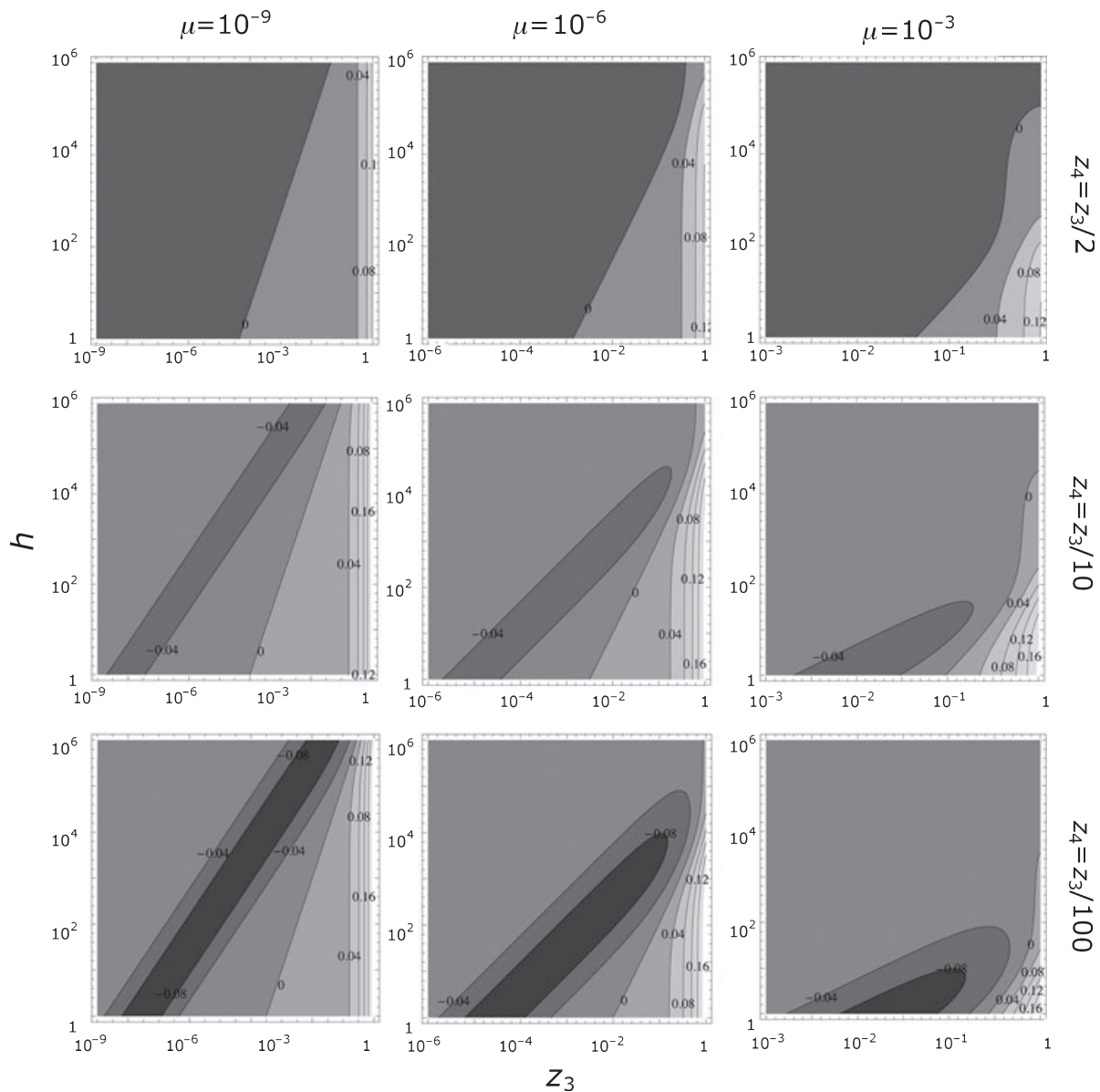
known and clear from the right part ( $s_3 \gg \mu$ ) of the plots with no juvenile selection in Fig. 2. Selection, therefore, must be neither too weak nor too strong for the evolution of robustness.

With juvenile soft selection, however, the results are quite different (Fig. 2). If the selection coefficients against the mutants are high enough, the antirobust allele  $x_1$  increases in frequency over the robust allele  $x_2$ , that is hypersensitivity to mutations (antirobustness) is favoured. Rather strong selection is necessary, but not high mutation rates: contrarily to the evolution of robustness, the magnitude of the mutation rate does not make any relevant difference for the evolution of anti-robustness. This is evident from Fig. 3: (i) strong selection ( $z_3$ ) against the mutants in the juvenile phase favours the evolution of antirobustness (whereas robustness is favoured at intermediate levels of selection); (ii) the ratio between juvenile and adult selection ( $h = z_3/s_1$ ) does not matter unless it is very high (i.e. unless selection in the adult phase is negligible) if the mutation rate  $\mu$  is not too high; at high mutation rates robustness evolves only if  $h$  is not too high (for robustness, instead,  $h$  always influences the ranges of selection coefficients); (iii) the ratio  $z_3/z_4$  (the relative fitness of the two mutants) does not matter unless the mutation rate  $\mu$  is very high (robustness, instead, evolves more easily if  $z_3/z_4$  is high); (iv) low mutation rates  $\mu$  allow the evolution of antirobustness for any value of  $h$  (i.e. even if selection in the adult phase is negligible) provided juvenile selection is strong; with higher mutation rates a weaker selection in the juvenile phase still leads to antirobustness, but a lower  $h$  is necessary (selection must also occur in the adult phase).

Note also that, with soft selection, the antirobust allele  $x_1$  can increase in frequency over the robust allele  $x_2$  even when the antirobust allele itself  $x_1$  (not only its mutant  $x_3$ ) has lower viability than the robust allele  $x_2$  in



**Fig. 2** Equilibrium frequencies of the four alleles with only hard ( $z_1 = 0$ ) or with hard and soft ( $z_1 = s_1$ ) selection;  $s_4/s_3 = 1/10$ ;  $s_1 = s_2 = 0$ .

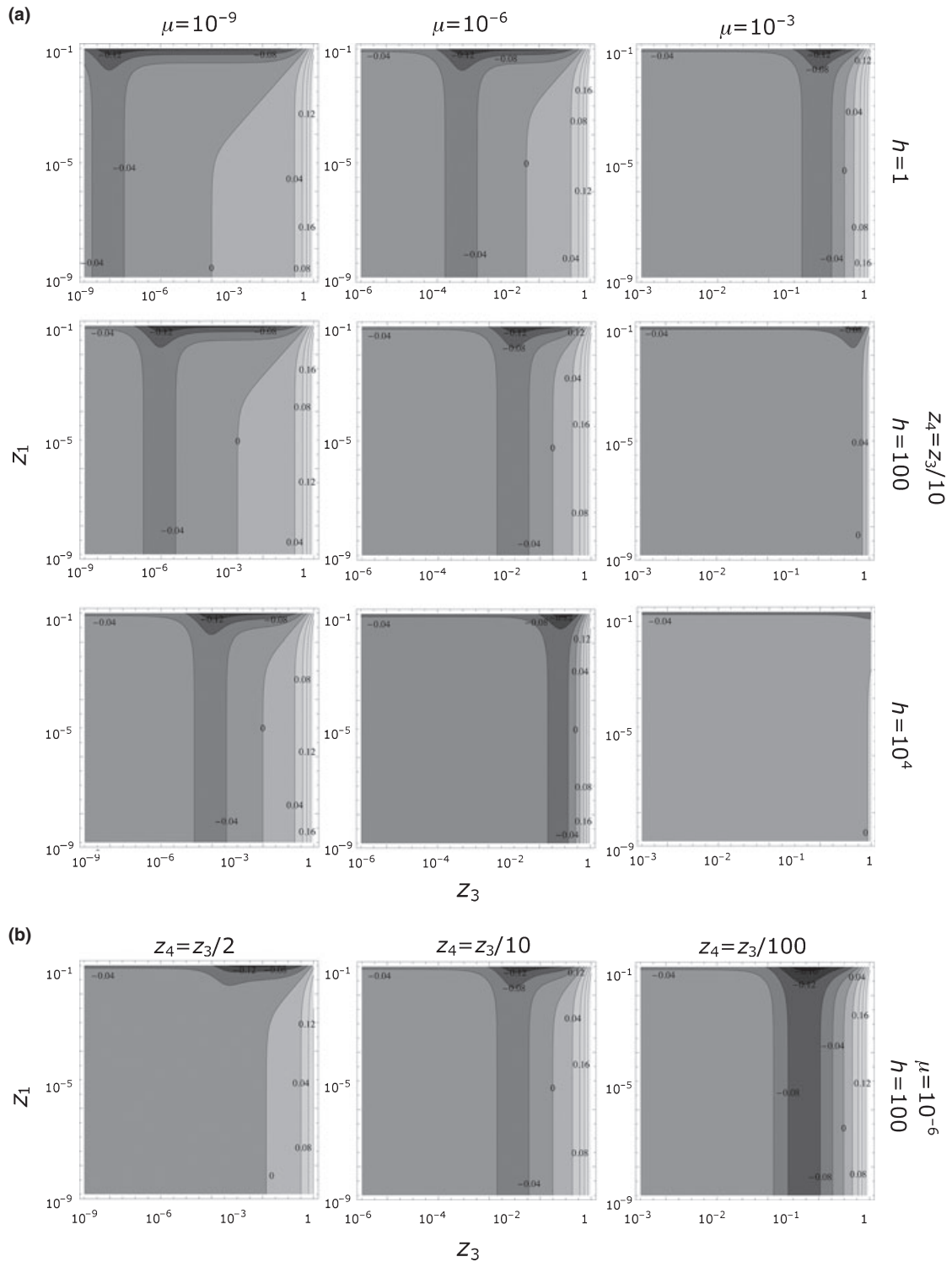


**Fig. 3** Contour plots of the difference in equilibrium frequency of alleles  $x_1$  and  $x_2$  as a function of  $z_3$  (the juvenile selection coefficient against the mutant  $x_3$  of the antirobust allele  $x_1$ ) and  $h$  (the ratio of juvenile and adult selection  $z_3/s_3$  and  $z_4/s_4$ : for  $h = 1$  selection in the adult phase is as strong as in the juvenile phase; for  $h \rightarrow \infty$  selection in the adult phase becomes negligible). Positive values indicate that the antirobust allele ( $x_1$ ) is more frequent than the robust allele ( $x_2$ ). Each plot corresponds to different values of  $z_4$  (the juvenile selection coefficient against the mutant  $x_4$  of the robust allele  $x_2$ ) and mutation rates  $\mu$ ;  $s_1 = s_2 = z_1 = z_2 = 0$ .

the juvenile stage (Fig. 4), provided selection against  $x_1$  is not too strong. If  $z_2 = 0$ , for example, the conditions for the evolution of antirobustness are virtually the same for any  $z_1 < 0.01$ ; only for higher values of  $z_1$  or if  $h$  is high (adult selection negligible) and  $\mu$  is very high, antirobustness does not evolve.

In many cases, therefore, with juvenile soft selection the antirobust allele will be more frequent than the

robust allele, even when the antirobust allele itself has a slightly lower viability. A high mutation rate is not necessary. The crucial point for the evolution of antirobustness is not the rate of back mutations, but the two-stage selection process: selection in the juvenile phase acts as a sieve against deleterious mutations; selection in the adult phase confers an advantage to an antirobust allele.



**Fig. 4** Contour plots of the difference in equilibrium frequency of alleles  $x_1$  and  $x_2$  as a function of  $z_3$  (the juvenile selection coefficient against the mutant  $x_3$  of the antirobust allele  $x_1$ ) and  $z_1$  (the juvenile selection coefficient against the antirobust allele  $x_1$  itself). Positive values indicate that the antirobust allele ( $x_1$ ) is more frequent than the robust allele ( $x_2$ ). Each plot in (a) corresponds to different values of  $h$  (the ratio of juvenile and adult selection  $z_3/s_3$  and  $z_4/s_4$ ; for  $h = 1$  selection in the adult phase is as strong as in the juvenile phase; for  $h \rightarrow \infty$  selection in the adult phase becomes negligible) and different mutation rates  $\mu$ ;  $s_4/s_3 = z_4/z_3 = 1/10$ . Each plot in (b) corresponds to  $h = 100$ ,  $\mu = 10^{-6}$  and different values of  $z_4$  (the juvenile selection coefficient against the mutant  $x_4$  of the robust allele  $x_2$ ). In both (a) and (b),  $s_1 = s_2 = z_2 = 0$ .

## The competition model

### Simulations

As Wilke *et al.* (2001) point out, direct evidence for a selective advantage of robustness must come from experiments in which a fitter genotype is displaced by a less fit but more robust one, when such organisms are put in competition against each other. In the present case, evidence for an advantage of antirobustness should come from competition experiments between two genotypes of which one has a higher sensitivity to mutations but the same fitness (or even lower fitness). I will use the same approach as Wilke *et al.* (2001) simulating competition of two genotypes with different degrees of robustness, evolving independently first, and in competition afterwards, and measuring the prevalence of the two genotypes in the final population after competition.

Consider two genotypes  $g = A, B$  starting with an equal number of deleterious mutations  $i = 0$ . At each generation, new deleterious mutations occur with a frequency  $u$  per genome per generation (the mutation rate per genome is divided by the number of genes and this is the probability that each gene can mutate per generation. This is necessary for  $u > 1$ ). The fitness of an individual with genotype  $g$ , is

$$W_g = V_g(1 - i/\eta)^{\kappa_g}$$

where  $V_g$  is the fitness of genotype  $g$  with no deleterious mutations; the magnitude of selection against the mutants is controlled by the parameters  $\eta$  (the number of mutations required to reduce fitness to zero) and  $\kappa_g$  (the degree of robustness:  $\kappa_g < 1$  indicates a robust strain,  $\kappa_g > 1$  an antirobust one). Individuals are chosen with a probability proportional to their fitness (individuals already chosen are not excluded from being chosen again); parents die after reproduction. Selection is always hard in the adult phase and soft selection can occur in the juvenile phase.

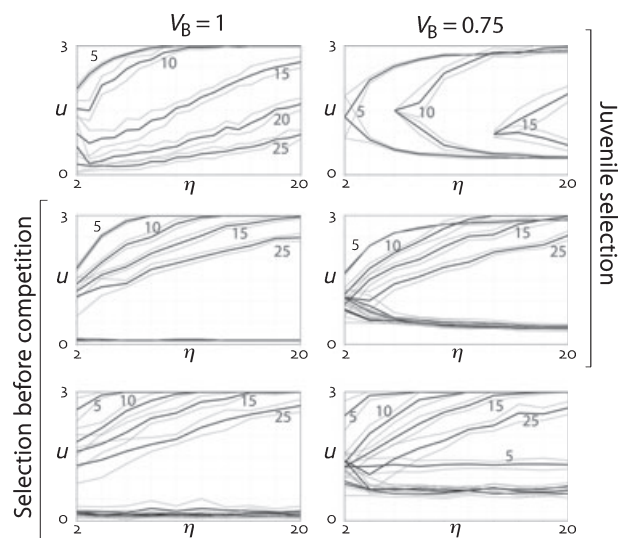
There are two stages in the simulations. In the first stage, I allow a sub-population of individuals of each genotype to evolve independently for a number of generations, not in competition with other sub-populations. This first stage (independent selection before the competition experiment) creates sub-populations in which the final distribution of mutations depends on the degree of robustness of the initial genotype. Since after the first stage individuals from both sub-populations are mixed at equal frequencies, this first stage is a soft selection phase. The difference with juvenile selection is that this first phase of selection occurs in different locations and the sub-population then migrate to a common location where competition occurs (see Levene, 1953; a similar scenario has been modelled by Wilke *et al.*, 2001 and O'Fallon *et al.*, 2007); moreover here multiple rounds of selection are possible. This first stage is not essential, and can be skipped, but it is useful to

understand that the crucial point for the evolution of antirobustness is the two-stage selection process: soft selection acting as a sieve against deleterious mutations followed by selection that confers an advantage to an antirobust allele. If this is true, independent selection within separate populations followed by a second stage of competition without soft selection should lead to similar results as juvenile soft selection followed by adult hard selection.

In the second stage, I mix individuals of two different populations (with different robustness) into a common population, at equal frequencies, to put them in competition with each other. These two population can have evolved separately previously (first stage – independent selection without competition) or not. I am interested in the final state of this mixed population, when the two competing genotypes have different degrees of robustness. This, therefore, is not a simulation for the evolution of antirobustness, it is clonal competition between more and less antirobust strains.

### Results

Some examples of the results for these different cases are shown in Fig. 5, in which an antirobust genotype B ( $\kappa_B > 1$  and  $V_B \leq 1$ ) is in competition against a more



**Fig. 5** The limit values (the black lines represent the average for 10 runs; the grey lines next to each black line are the average  $\pm$  standard deviation) of  $u$  (genomic mutation rate) for which the antirobust strain (B) completely displaces the robust one (A) in the simulated competition experiment for different values of  $\eta$  and  $\kappa_B$  ( $\kappa_B$  is indicated next to the maximum limit value of  $u$  within each plot). The minimum limit values of  $u$ , very close to each other, are not labelled for clarity. Population size: 1000; number of offspring: 10;  $V_A = 1$ ;  $\kappa_A = 1$ ; juvenile selection only in the first two rows; independent selection (50 generations) before the competition experiment only in the second two rows.

robust genotype A ( $k_A = 1$  and  $V_A = 1$ ). With no soft selection and with no independent selection before the competition experiment, the antirobust genotype is always eliminated (not shown in Fig. 5). The antirobust genotype, however, replaces the robust genotype if the competition experiment is preceded by independent selection within the genotype or (in the absence of independent selection before competition) if during the competition stage there is soft selection in the juvenile phase (or if both soft selection and independent selection before competition occur – Fig. 5). Selection within separate sub-populations followed by competition, therefore, may lead to the same results as soft juvenile selection followed by hard selection: in both cases, the first phase (selection within a sub-population or juvenile soft selection) acts as a sieve against deleterious mutants.

There is an upper value of the genomic mutation rate  $u$  that limits the stability of antirobustness; this value decreases with  $\kappa_B$  and increases with  $\eta$ , that is, it is lower for higher degrees of antirobustness. This is because when the mutation rate is very high some of the mutant offspring of the antirobust genotype will manage to go on to the adult phase, and here the deleterious effect of their allele will make the antirobust genotype less competitive. In general, with soft selection robustness can still prevail at very high mutation rates, but antirobustness will prevail for a reasonable range of parameters.

## Discussion

I have shown that soft selection during the juvenile phase can favour the allele (antirobust) whose mutants are more sensitive to the effects of deleterious mutations, even if this allele itself does not have higher viability or reproductive success. Indeed, as I have shown, selection may even favour alleles that are simultaneously antirobust and slightly maladaptive in the juvenile phase.

The results of the model suggest that antirobustness can evolve under realistic, low mutation rates. Selection against the mutants should be rather strong in the juvenile phase and selection, even if weak, must also occur in the adult (at high mutation rates, instead, antirobustness evolves only if both juvenile and adult selection are strong). Therefore, it is likely that antirobust genes are those that have a crucial role in the juvenile phase and have also consequences in the adult, e.g. genes necessary for correct development or genes involved in basic cellular functions (this seems to be the case for the most antirobust genes in *Drosophila melanogaster* – see Table 3 in Archetti, 2006), but not genes expressed only in the adult, nor genes expressed exclusively in the juvenile phase.

My analysis could be extended to diploids. Here, we might find different effects for mutations that are recessive. Buchholz (1922) and Klekowski (1988) discuss how soft selection could affect the spread of recessive deleterious alleles by discarding homozygous juvenile

individuals, but they do not discuss the evolution of robustness and antirobustness that I analysed here. Moreover, my model is a model of auto-robustness and it would be interesting to see whether a modifier approach would give similar results, although I see no reasons why the results should be different, at least in a haploid model.

## Developmental selection

My definition of soft selection has remained vague, encompassing all cases in which there is competition for a determined number of biological spaces. Sib-competition in a nest is a possible example: in many birds the number of eggs that hatch is larger than the number of nestlings that the parents can take care of; usually only a few of the young birds survive the juvenile phase, in which they compete with each other for limited food from the parents. Soft selection in a clutch, however, is probably not strong enough to provide the right coefficients described in my model. This is not the only possibility, however, and here I extend this concept to more general cases.

It is not necessary that competition occurs in the external environment. It can also occur in the internal environment of the organism. Competition among sperms within an individual (not sperm competition between individuals) is an example of soft selection, because the initial number of sperms is large compared to the number of sperms (one) that can go on to fertilize the egg; even discarding most of them does not affect viability (this is why, incidentally, meiotic drive is usually effective only in males). Although I will neither discuss selection among sperms nor germline selection, juvenile and developmental selections alter the transmission of alleles in a way analogous to meiotic drive.

Buchholz (1922) was the first to note that soft selection could operate in the internal environment of the organism, and he coined the term ‘developmental selection’ to describe it. The term was used mainly in relation to plants, because many plants have life cycle characteristics that generate one or more stages of soft selection. Soft selection can be found in some form in the life cycle of practically all vascular plants, and also in many cryptogamic forms and in animals (Buchholz, 1922; Klekowski, 1988).

Pollen (microgametophyte) competition was proposed already by Buchholz (1922) and has been reported in some cases (Jones & Tippo, 1952; Meinke, 1985; Klekowski, 1988). It is in the megagametophyte, however, that soft selection is more evident. Ovule ontogeny has a number of levels in which competition and selection may occur, because the megagametophyte (embryo sac) may develop from more than one megaspore (at least in angiosperms) and more than one megagametophyte may form per ovule (Maheshwari, 1950). Soft selection is possible because one cell in the megagametophyte

ultimately functions as the egg, and one embryo matures per ovule. In monosporic megagametophytes, however, soft selection is possible only if the choice of the single megaspore that will give rise to the embryo sac is not rigidly spatially determined within the ovule, as it happens for example in the *Oenothera* type (sensu Maheshwari, 1950). Because the haploid nuclei undergo a number of mitotic divisions in the same embryo sac, mutants can arise that are in competition with each other, exactly as in my simple model of soft selection (Fig. 1). In bisporic or tetrasporic megagametophytes (originated from more than one megaspore), there is a further possibility of soft selection because competition may occur not only between megagametophytes but also between embryos.

The formation of multiple embryos within a single ovule is a significant aspect of developmental selection in plants. Polyembryony is common in plants, and both true (multiple embryos formed by a single megagametophyte) and false (multiple embryos formed by different megagametophyte) polyembryony may give rise to soft selection, because generally one embryo per ovule survives to produce the sporophyte. False polyembryony, as discussed above, gives rise to actually two stages of soft selection, because it allows competition between megagametophytes too.

Soft selection during embryonic competition may occur in mammals during gestation and development (Haig, 1996). Soft selection at the level of polyembryonic broods, also appears to be responsible for eusociality in certain parasitoid wasps – i.e. the existence of a sterile ‘soldier’ caste that mediates sex ratio conflict between siblings – and it has been suggested that the soldiers may attack mutated embryos in order to improve the quality of the brood (P. Nonacs, as reported by Gardner *et al.*, 2007).

Further possibilities for soft selection exists in flowering plants. Generally seed plants produce more ovules than seeds (Wiens, 1984): this allows competition between unfertilized ovules within an ovary and those megagametophytes developing from megaspores with deleterious mutations may cause the ovule to abort; competition between fertilized ovules within an ovary, on the other hand, may allow selection for the most viable embryos. Seed plants also produce more flowers than fruits (Stephenson, 1981), and it was Darwin (1883) himself to suggest that this may enable plants to regulate the quality of their offspring through selective fruit abortion (see also Janzen, 1977; Charnov, 1979; Lee & Bazzaz, 1982; Stephenson & Winsor, 1986).

Competition between spores, gametophytes, embryos or ovules, as in the examples described above, is always stronger when the competing elements develop simultaneously. Delay of fertilization or embryo maturation may be a way to synchronize competing elements and allow a more efficient selection among competing individuals. Willson & Burley (1983) discuss this idea in relation to

sexual selection in plants, but the argument is essentially the same for selection between embryos, ovules or gametophytes.

In summary, it seems that, especially in plants, but also in animals, there are many stages that may function as soft selection sieves.

### Antirobustness

Buchholz (1922) and subsequent plant biologists (e.g. Klekowski, 1988) discussed how soft selection could affect the spread of recessive deleterious alleles by discarding homozygous juvenile individuals. They did not discuss the evolution of robustness and antirobustness. Cases of antirobustness, however, have been discussed in a variety of other situations, ranging between very general, e.g. incomplete dominance or haploidy (de Visser *et al.*, 2003) to very specific, e.g. overlapping reading frames (Krakauer, 2000), checkpoint genes inducing apoptosis such as p53 (Lowe *et al.*, 2004) and synonymous codon usage (Archetti, 2006). Krakauer & Plotkin (2002) suggest that antirobustness is more likely in large populations for removing deleterious mutants, whereas small populations will be more robust to ensure that all individuals have a high chance of survival (see also Elena *et al.*, 2007).

O’Fallon *et al.* (2007) show that populations divided into discrete patches connected by dispersal may also favour antirobust genotypes. This is similar to Levene’s (1953) metapopulation model, and a similar scenario (populations evolving separately before competing) is described in my competition simulation, where I show that the effects of juvenile soft selection are similar to the effects of independent selection within sub-populations before competition. O’Fallon *et al.* (2007) are concerned primarily with microbes, in particular RNA viruses, whereas my model applies to multicellular organisms. There is a similarity with the model of O’Fallon *et al.* (2007), however, in that we both have two stages of selection: in O’Fallon *et al.* (2007) because of migration between different subdivided populations, in which selection occurs first within a group and then in the whole population; in my model instead selection occur first during development and then in the adult phase, with no migration and no subdivided populations. The model of O’Fallon *et al.* (2007) is set in the context of the standard quasi-species model, whereas my model is rather a model of intragenomic conflict. Finally, in my model, hypersensitivity to mutations can evolve even if it is slightly deleterious. This is not discussed by O’Fallon *et al.* (2007) but it cannot be excluded that this possibility could arise in their scenario.

It is important to point out that, according to my model, antirobustness may evolve despite conferring no immediate advantage to the individual but in certain cases even if it entails a lower viability in the juvenile phase. This can lead to a kind of intragenomic conflict. In

fact, from the point of view of the individual, an antirobust allele is deleterious because it leads to more deleterious mutations in the progeny; but from the point of view of the gene itself, being hypersensitive to mutations is the optimal strategy when its bearer undergoes soft selection, because it will get rid of its mutants more easily and pass to the adult phase (and therefore to the next generation) more copies of the original gene.

Antirobustness is maladaptive from the point of view of the organism also because, even if it does not affect its viability or if it does not entail any negative juvenile effect, genes that are hypersensitive to mutations will end up accumulating somatic mutations that may lead to more dramatic deleterious effects at a later age, like genetic diseases and cancer. Somatic mutations that occur during early development are thought to be responsible for susceptibility to cancer. It is possible that somatic mutations in anti-robust genes may lead more easily to genetic diseases including cancer. Understanding the dynamics of antirobustness in cell populations and detecting antirobust genes, therefore, may be extremely important also for our understanding of genetic diseases.

Selection within a clutch and possibly within the uterus of the mother was also discussed in the framework of the 'selection arena' (Stearns, 1987) and of the 'tangled bank' hypothesis, which is in essence a sib-competition argument for the evolution of sex (Bell, 1982). While this previous work does not address the evolution of robustness and antirobustness, robustness and antirobustness can be important factors in the evolution of sex. Models of synergistic epistasis (Kondrashov, 1982, 1988), e.g. put much emphasis on the effect of getting rid of deleterious mutations, and soft selection could probably make these models work more efficiently (Peck & Waxman, 2000).

More in general, it seems that many stages of the life cycles especially in plants, but also in animals, allow soft selection to operate. This may lead, as I have shown, to an increase in frequency of alleles that are hypersensitive to the effects of deleterious mutations, that is to an advantage for genotypes located in steeper regions of the fitness landscape ('survival of the steepest') even if these genotypes occupy slightly lower fitness peaks.

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