



## Influence of the rate of introduction on the fitness of restored populations

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### Abstract

Most studies using demographic PVA models in a context of species restoration have concluded that rather than the rate of introduction, the total number of individuals released had the most important significant influence on the chance of success. In this article we use a genetic simulation model including deleterious and adaptive alleles to assess the impact of the method of release on the change in population mean fitness. We systematically compare a strategy that consists in releasing all individuals at the same time with a strategy that consists in staggering releases over a long period of time. Our results show that the former strategy is more beneficial for long-term fitness when considering advantageous genes only, while the latter is better when considering deleterious genes only. If deleterious and adaptive alleles are considered together, the best strategy depends then essentially on which of these types of alleles has the stronger influence on the change in total fitness. Although the relative contributions of the variance in total fitness due to adaptive and deleterious alleles may vary with the initial frequencies and the selective and dominance effects of these alleles, our results show that the optimal rate of release is mostly dependant on the expected long-term population size. Thus from a genetic view-point, the temporal release strategy of reintroduced populations should be considered with respect to their environment's carrying capacity.

### Introduction

Short- and long-term viability is reduced in small populations by two well studied genetic processes: inbreeding depression and loss of genetic variability (Lande 1988). On one hand, the reduction of viability and fecundity known as inbreeding depression is caused in part by the increasing homozygosity of numerous slightly detrimental mutations which may also become fixed despite counteracting selection, and accumulate (Morton et al. 1956; Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Lynch et al. 1999). Another part of inbreeding depression is due to individually rare but collectively abundant, nearly recessive lethal or semi-lethal mutations (Simmons and Crow 1977; Lande 1988; Crow 1993; Wang et al. 1998). On the other hand, genetic drift tends to

reduce genetic variation, leading eventually to the loss of adaptability to environmental changes (Fischer 1930; Gilpin and Soulé 1986). Reintroductions and reinforcements are likely to become an increasingly important tool for the maintenance of demographically and genetically viable populations (May 1991). Reintroduction success is expected to be strongly linked to the genetic problems mentioned above. On one hand, restoration projects that involve the release of few individuals issued from large natural populations imply a precipitous reduction of population size, which tends to engender a strong inbreeding depression because selection has not the opportunity to rid the population from deleterious alleles (Soulé 1980; Lande 1988). On the other hand, by definition a reintroduction follows the extinction of the locally adapted natural population (IUCN 1998).

Thus, it implies releasing individuals that are locally ill-adapted. The viability of such population depends then mostly on its ability to adapt to its new environment, which is positively related to the extent of genetic variation retained within the population. The genetic composition of restored populations is of major importance since it constitutes the stock on which selection may act to produce locally adapted genotypes. In order to allow the best adaptation of the released population to its new environment, two strategies have been proposed. The first consists in releasing individuals from the populations most likely to have local adaptations to the release site, such as indigenous populations (Montalvo and Ellstrand 2000). Such approach is not always possible, due to a lack of appropriate individuals, and it involves the risk of releasing a genetically low variable population. The alternative strategy is to release individuals from a variety of populations in order to maximise the genetic variability on which selection will act (Tordoff and Redig 2001).

While the question of the number and provenance of the individuals to release has been addressed in several studies aiming at identifying optimal reintroduction strategies (Green 1997; Helenurm 1998; Wilkinson 2001), the temporal organisation of releases has received little attention, especially from a genetic view-point. This study aims to assess the influence of the rate of release on the fitness evolution of a restored population in the case where founders are *a priori* ill-adapted to their new local environment and exhibit a high genetic variability. For that purpose, we use a simulation model including both genetic and demographic considerations, and we systematically compare the relative efficiencies of two release strategies on fitness. The first strategy consists in releasing all individuals at the same time ("punctual release") and the second one consists in staggering the releases over a long period of time ("progressive release"). We examine the influence of different types of selected genetic variation on population fitness according to these two strategies, and how they interact with various factors that can be estimated or controlled by population managers (i.e., number of released individuals, duration of the release period, population replacement rate, carrying capacity of the release area).

## Methods

### *Stochastic model-life cycle*

We use a two-sex individual-based model with non-overlapping generations and one age class. Demographic stochasticity is not considered and all individuals present at a given time pair randomly, so effective population size equals real population size. Each generation, parents pair and the survival of the offspring (that depends on its genotype) is the only parameter of fitness. Survival events occur through Bernoulli trials. Population size  $N_t$  at generation  $t$  is not stochastically determined and depends only on the number of released individuals ( $N_r$ ), the release strategy, the net replacement rate  $R$ , and the carrying capacity  $K$ . At generation  $t$ , reproduction ends when the number of surviving offspring reaches  $\text{Min}(R \cdot N_{t-1}, K)$ , and the cohort of reproducers disappears.

In all cases, we assume that all released individuals descend from large outcrossing populations. The punctual release is obtained by releasing the overall specified number of founders  $N_r$  at generation 0, and the progressive release is obtained by staggering releases at a constant rate per generation within  $d$  generations. The total number of released individuals is the same for the two approaches. All comparisons between release strategies are conducted for different combinations of the parameters  $d$  (ranging from 5 to 50),  $K$  (from 25 to 500), and  $N_r$  (from 5 to  $K$ ). Two scenarios were investigated with respect to the net replacement rate of the population  $R$ . The "high" replacement rate corresponds to an infinite growth rate to the carrying capacity  $K$  (that means that the population size is constant and equal to  $K$ ) and the "low" replacement rate corresponds to a net replacement rate of 1.5 per generation.

### *Stochastic model-genetic aspects*

The genome of each individual is described as three series of 150 different diploid loci. Each of these three series can carry two types of alleles at each locus: a wild-type and a deleterious/beneficial allele. Each series corresponds to a given type of selected alleles (mildly deleterious, lethal and adaptive). The probability of transmission of a given allele is then dependent upon its selective effect and its coefficient of dominance but is also influenced by the background variance in fitness caused by other segre-

gating loci on which selection acts. As all released individuals descend from large outcrossing populations, the expected initial frequencies of mildly deleterious and lethal alleles are given by the mutation-selection balance. Using these mean frequencies, the initial number of each type of deleterious alleles present in each founder is then stochastically determined from a Poisson distribution. The probabilities of transmission of alleles at each locus during the fertilisation are given by the Mendelian rules (Bernoullian process). New deleterious mutations stochastically occur in each diploid genome (Poisson distributed). Genetic parameters used for deleterious alleles (i.e., selective and dominance coefficients, and mutation rates) correspond to values commonly assumed for mildly deleterious and lethal mutations (Simmons and Crow 1977; Lande 1995; Drake et al. 1998; Lynch et al. 1999). However, due the lack of estimates of such parameters for adaptive mutations, we investigate broad ranges of values for the selective and dominance coefficients of these mutations. All genetic parameters are given in Table 1. We assume multiplicative interactions for fitness and free recombination of all loci. Thus, the presence and the accumulation of alleles is characterised in terms of survival probability of each newborn individual  $i$ , by using a genetic factor  $w_i$ , calculated as

$$w_i = (1 - h_d \cdot s_d)^{nd1} \cdot (1 - s_d)^{nd2} \cdot (1 - h_l \cdot s_l)^{nl1} \cdot f_{hl}(nl2) \cdot (1 + h_{ad} \cdot s_{ad})^{nad1} \cdot (1 + s_{ad})^{nad2}$$

where  $s_d$ ,  $h_d$ ,  $s_l$ ,  $h_l$ ,  $s_{ad}$ ,  $h_{ad}$  are the selective and dominance coefficients for detrimental, lethal and adaptive alleles;  $nd1$ ,  $nl1$ ,  $nad1$  are the numbers of detrimental, lethal and adaptive alleles present at the heterozygous state in individual  $i$ ;  $nd2$ ,  $nl2$ ,  $nad2$  are the numbers of detrimental, lethal and adaptive alleles present at the homozygous state in individual  $i$ ; and  $f_{hl}$  is the function “homozygous

lethal”, defined by  $f_{hl}(0) = 1$  and  $f_{hl}(x) = 0$  for any  $x \neq 0$ . Changes in relative fitness are investigated in several scenarios of reintroduction by using Monte Carlo simulations in which 1000 population trajectories are drawn.

#### Deterministic equation for neutral variation

In order to assess the loss of genetic diversity in the simple case where there is no selection, we also use a simple equation for the evolution of inbreeding in the restored population. In this case, we investigate only two extreme situations with respect to the replacement rate ( $R = \infty$  and  $R = 1$ ). Progressive releases are modelled using an immigration rate  $m$ . We use the following equation for the inbreeding at time  $t$ :

$$f_{(t+1)} = [1/2N_{(t)} + (1 - 1/2N_{(t)}) \cdot f_{(t)}] \cdot (1 - m_{(t)})^2$$

where  $N_{(t)}$  and  $m_{(t)}$  represent, respectively, the population size and the immigration rate at time  $t$ . In all cases, we assume that the number of released individuals equals the carrying capacity ( $N_r = K$ ). Therefore, for punctual releases,  $N_{(t)}$  and  $m_{(t)}$  are given by:

$$N_{(t)} = N_r = K \quad \forall t \\ m_{(t)} = 0 \quad \forall t$$

For staggered releases with a high replacement rate ( $R = \infty$ ),  $N_{(t)}$  and  $m_{(t)}$  are given by:

$$N_{(0)} = N_r/d \\ N_{(t)} = N_r = K \quad \text{for } t \neq 0 \\ m_{(t)} = 1/d \quad \text{for } t < d \\ m_{(t)} = 0 \quad \text{for } t \geq d.$$

For staggered releases with a low replacement rate ( $R = 1$ ),  $N_{(t)}$  and  $m_{(t)}$  are given by:

Table 1. Genetic parameters used in the model

Type of mutation	Coefficient of selection	Coefficient of dominance	Genomic rate of current mutations	Initial frequency
Lethal	1 <sup>a</sup>	0.02	0.05	Mut-sel balance
Mildly deleterious	0.05 <sup>a</sup>	0.3	1	Mut-sel balance
Adaptive	0–0.5 <sup>b</sup>	0–1	0	0.005–0.1

<sup>a</sup>Fitness reduction.

<sup>b</sup>Fitness improvement.

$$\begin{aligned}
 N_{(0)} &= N_r/d \\
 N_{(t+1)} &= N_{(t)} + (N_r/d) \quad \text{for } 0 < t < d \\
 N_{(t)} &= N_r = K \quad \text{for } t \geq 0 \\
 m_{(t)} &= N_r/(d \cdot N_{(t)}) \quad \text{for } t < d \\
 m_{(t)} &= 0 \quad \text{for } t \geq d.
 \end{aligned}$$

## Results

### Neutral genetic variation (deterministic equation)

A comparison between punctual and progressive release strategies indicates that staggering releases over time has a beneficial effect on the maintenance of neutral genetic variation whatever the growth rate and the total number of releases (Figure 1). When considering the dynamic aspect of the preservation of genetic variability, it appears

that progressive releases allow to reach the same level of genetic variability as punctual releases with about half the number of founders. As the effective rate of immigration induced by progressive releases is always higher when the growth rate of the population is low, the maximum genetic diversity is paradoxically obtained for populations with the low replacement rate.

### Selected variation (stochastic model)

All comparisons between release strategies have been conducted for different combinations of the parameters  $d$ ,  $K$ , and  $N_r$ . The time over which releases occur with the progressive strategy ( $d$ ) has no notable effect. However, as expected, as  $d$  decreases, the difference between the two strategies becomes smaller. Similarly, as the overall number of founders ( $N_r$ ) decreases, short- and long-term fitness decreases with the two strategies, but with

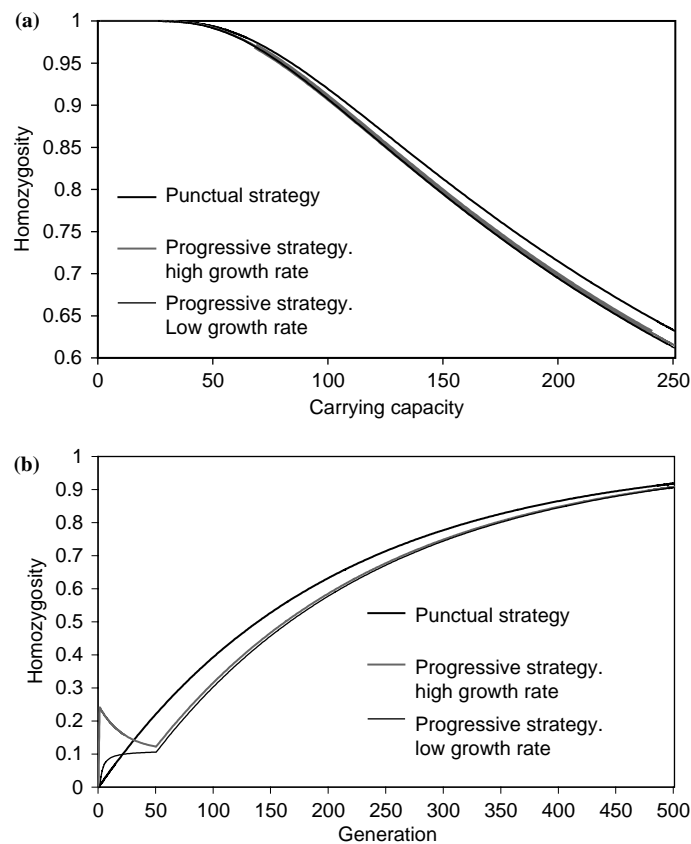


Figure 1. Loss of neutral genetic variation resulting from the two release strategies (deterministic equation). In all cases,  $d = 50$ . (a) after 500 generations. In all cases,  $N_r = K$ ; (b)  $N_r = K = 100$ .

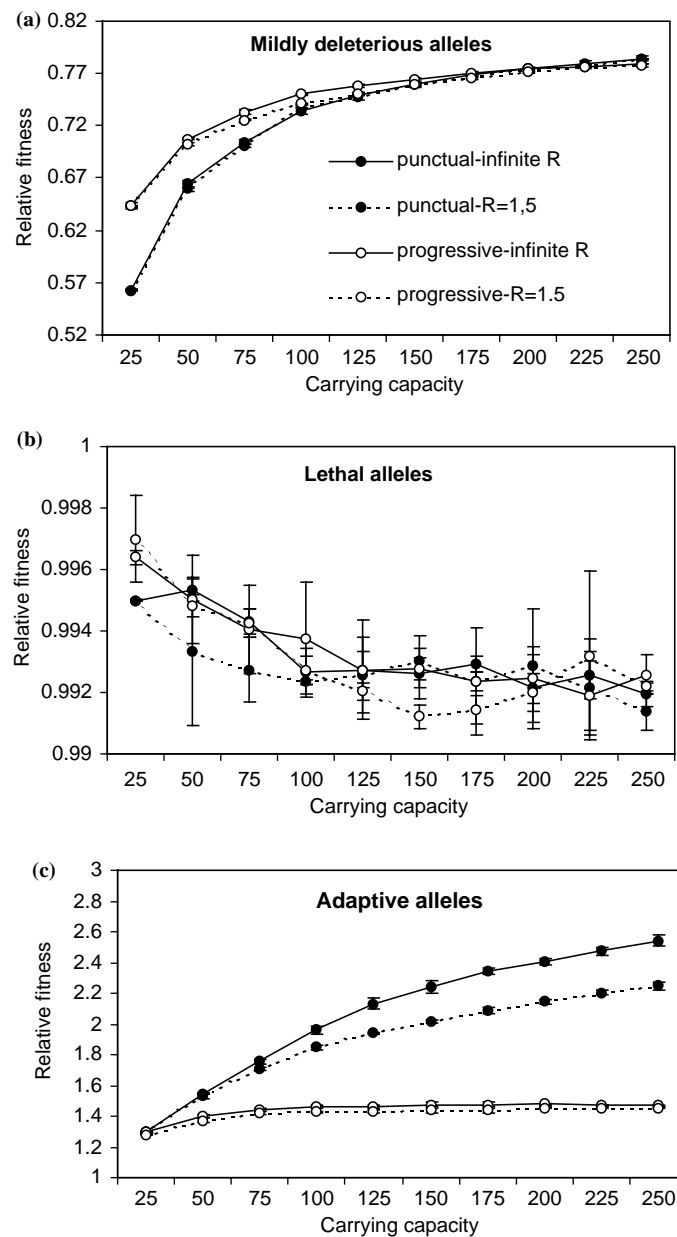


Figure 2. Relative fitness after 100 generations as a function of the carrying capacity. In all cases,  $N_r = 25$ . Error bars represent standard error of the mean. (a) model including lethal alleles only; (b) model including mildly deleterious alleles only; (c) model including adaptive alleles only, with  $s_{ad} = 0.1$ ,  $h_{ad} = 0.5$ ,  $q_{0ad} = 0.05$ . Results obtained after 100 generations.

no substantial effect on their relative efficiencies, for any combination of all parameters. Hence, as the relative efficiencies of the two release strategies were primarily sensitive to  $K$ , Figures 2–5 present results for fixed values of  $d$  and  $N_r$ . Figure 2 presents a comparison of the long-term relative fitness obtained with the two strategies of release, for

three categories of selected alleles. These three types of alleles are considered separately in three distinct models. When considering lethal genes, there is no significant difference among release strategies. Long-term fitness is close to 1 in all cases and essentially depends on the carrying capacity  $K$ . For mildly deleterious genes,

progressive releases lead to a higher fitness than punctual releases whatever the number of released individuals (not shown) and carrying capacity. By contrast, when considering beneficial genes, on a long-term scale the punctual release strategy is more advantageous than the progressive one and increasing the carrying capacity has almost no benefit on fitness when releases are spread over time. However, the coefficient of dominance of beneficial genes has also an influence on these results (Figure 3). In terms of its long-term effect on fitness, the punctual strategy is better than the progressive one when considering additive, partially recessive and recessive genes. On the contrary, the progressive strategy is more efficient when considering dominant and partially dominant genes. Simulations have been conducted using different values of the selective coefficient  $s_{ad}$ , leading to faster fitness improvements with high values of  $s_{ad}$  with both strategies. However,  $s_{ad}$  has no qualitative impact on the relative efficiencies of release strategies. For the three categories of mutations considered, although we have compared two extreme growth rates, we detect little impact of the growth rate on fitness changes, compared with the influence of the release strategy.

When considering jointly the impacts of deleterious (i.e. detrimental + lethal) and adaptive mutations, results diverge from those presented above due to a substantial interplay between these different functional categories of mutations. Although all loci are assumed to be independent from each other in terms of selective effect and probability of transmission, selection may generate some

interference between them. When examining population trajectories with the progressive strategy, a negative correlation between the contributions of mildly deleterious and adaptive genes on individual fitness is detected (i.e., resident individuals who carry the highest frequencies of adaptive mutations are those who carry the highest frequencies of mildly deleterious mutations). This relationship is significant both within and among trajectories, for  $t < d$ , for any value of  $N_r$  and  $K$  comprised between 25 and 500 individuals. For the punctual release, no significant correlation was found.

The impact of this interaction is illustrated on Figure 4, which presents the long-term relative contributions of adaptive genes to fitness in the presence/absence of deleterious mutations. The incorporation of deleterious alleles into the model significantly improves the fitness gain due to adaptive alleles.

When the influences of deleterious and adaptive alleles are considered together, the relative efficiencies of the two release strategies may vary with several genetic and demographic parameters. When harmful mutation parameters are fixed and correspond to average values commonly assumed for nearly additive mildly deleterious mutations and lethal mutations (Simmons and Crow 1977; Lande 1988; Drake et al. 1998), the optimal release strategy depends both on the carrying capacity  $K$  and on the mean initial frequency of adaptive mutations  $q_{0ad}$ . These two parameters primarily determine the relative contributions of the two categories of alleles to the total temporal variance in fitness. If  $K$  is very low ( $< 50$  individuals), the progressive release

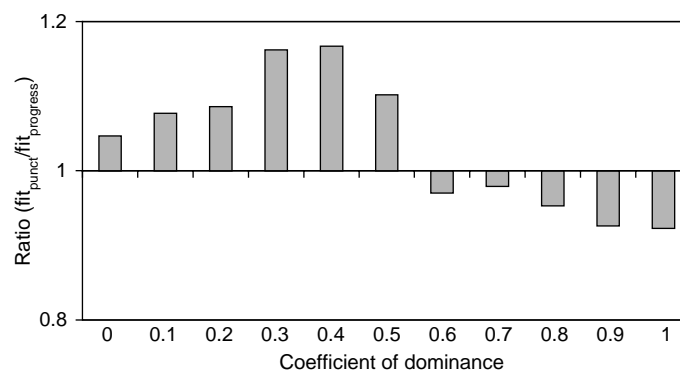


Figure 3. Relative efficiency of the punctual strategy compared to the staggered strategy (with  $R = \infty$ ) in terms of increase of the mean relative fitness due to adaptive alleles as a function of the coefficient of dominance of these alleles. Only adaptive alleles are considered. The strategies are compared after 100 generations.  $N_r = K = 100$ ,  $s_{ad} = 0.1$ ,  $q_{0ad} = 0.05$ .

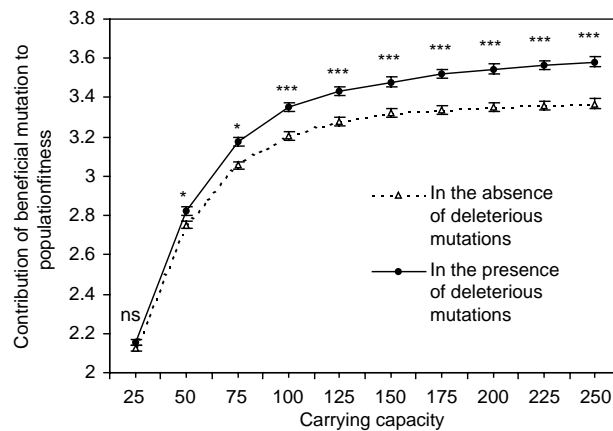


Figure 4. Relative contribution of beneficial mutations to overall population fitness after 100 generations. Progressive release with  $R = \infty$ ;  $N_r = 50$ ;  $d = 50$ ; for beneficial mutations,  $s_{ad} = 0.1$ ;  $h_{ad} = 0.5$ ;  $q_{0ad} = 0.033$ .  $t$ -tests were performed on 1000 independent trajectories to investigate the effect of deleterious mutations on the contribution of adaptive alleles on fitness ( $*P < 0.05$ ;  $***P < 0.0001$ ; ns, not significant). Error bars represent standard error of the mean.

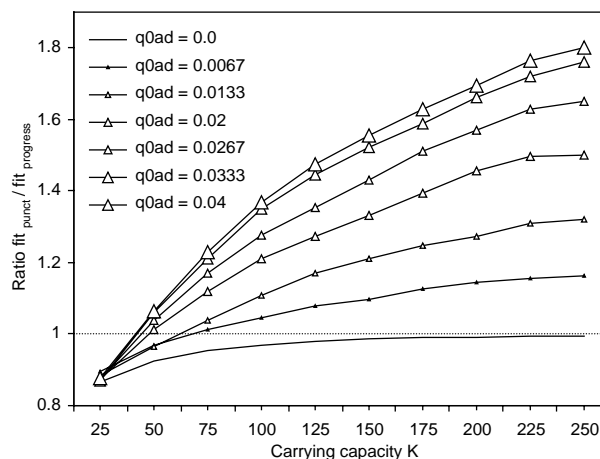


Figure 5. Relative efficiency of the punctual strategy compared to the staggered strategy (ratio of total mean fitness obtained with the punctual strategy on the mean fitness obtained with the progressive strategy with  $R = \infty$ ). Detrimental, lethal and beneficial mutations are considered and their contributions to fitness are assumed to interact multiplicatively. In all cases,  $N_r = 25$ ;  $d = 50$ ; for beneficial mutations,  $s_{ad} = 0.1$ ;  $h_{ad} = 0.5$ ;  $q_{0ad} = 0.033$ .

strategy is always optimal because the main temporal variance in total fitness is due to detrimental. If  $K$  is larger, the progressive strategy is optimal only if  $q_{0ad}$  is low (Figure 5).

## Discussion

### *Influence of selected genetic variation on fitness*

Several studies using demographic population viability analysis in a context of species restoration

have concluded that rather than the rate of introduction, the total number of individuals released had the most important significant influence on the chance of success (McCarthy 1994; Legendre et al. 1999; Sarrazin and Legendre 2000). However, in natural conditions, temporal fluctuations in demographic rates due to environmental stochasticity tend to improve the efficiency of a restoration with several release events relative to one single release event (Griffith et al. 1989; Haccou and Iwasa 1996). Our results, focussing only on genetic aspects, suggest that the rate of

introduction has a strong influence on the change in mean population fitness, which may have an important impact on long-term persistence. Further, from a genetic point of view, the best method of release depends largely on long-term population size, which determines partly the type of mutations that contributes the most to the temporal change in total fitness.

When mildly deleterious genes are considered, the progressive release is advantageous because it engenders a continuous gene flow, which is sufficient to counter-balance the accumulation of mildly deleterious alleles occurring within the population (Newman and Tallmon 2001; Couvet 2002). Although non-inbred newcomers represent a small proportion of the population each generation, their contribution to the gene pool (i.e., effective migration rate) is substantial, due to their higher fitness and to the beneficial effect of hybridization (Charlesworth and Charlesworth 1999; Ebert et al. 2002). The difference between the two strategies increases as the long-term population size decreases because the degree to which staggered releases (acting as a constant immigration rate) restore heterozygosity is dependant upon the rate of loss of the genetic diversity. By contrast, because there is no possible accumulation of lethal mutations over several generations due to rapid purging (Falconer 1989; Hedrick 1994; Kirkpatrick and Jarne 2000), they contribute almost nothing to heterosis (Whitlock et al. 2000) and no substantial difference between strategies has been detected on a long-term scale when lethal mutations only were considered. If adaptive genes only are considered, in the case of the progressive release, non-adapted newcomers exhibit on average a lower fitness than resident individuals issued from several generations of local selection. Their contribution is then dramatically reduced, impairing the arrival of new adaptive mutations into the population. In such a case, adaptation can only act on a reduced proportion of the genetic pool (the earlier released individuals). In terms of long-term effect on fitness, the punctual strategy is better than the progressive strategy when considering additive, partially recessive and recessive genes. This is because staggering releases tends to delay the action of selection on the pool of adaptive genes entering the population, which is particularly slow when considering recessive favourable genes (Maynard Smith 1989). At the opposite, the pro-

gressive strategy is more efficient when considering dominant and partially dominant genes. This is due to the higher allelic diversity retained within the population if releases are staggered over several generations (Figure 1). A high level of allelic diversity has a more beneficial impact on current fitness when good genes are dominant or partially dominant, due to the rapid action of selection.

If deleterious (lethal + detrimental) and adaptive genes are considered together, the process is substantially altered by some interference between detrimental and adaptive mutations. On one hand, individuals descending from several generations of selection in the local population exhibit both a strong adaptation and a high genetic load due to the accumulation of detrimentals. On the other hand, newcomers have no genetic load and are not adapted to the local site. At the population level, this divergence engenders a negative correlation between the contributions of detrimental and adaptive mutations to individual fitness, which reduces overall fitness variance among individuals. The efficiency of selection for removing detrimental alleles and for increasing the frequency of beneficial alleles is consequently affected when these two categories of alleles act together. Paradoxically, this interference is beneficial in terms of long-term adaptation, because newcomers contribute more to the next generation in the presence of detrimental genes than in their absence, so the arrival of new adaptive mutations is improved (Figure 4).

On a long-term scale, when deleterious and adaptive genes are considered separately, the model leads to two main results. On one hand, the strategy consisting in spreading the releases over a long period of time is advantageous when deleterious alleles are present. On the other hand, one single release event is advantageous when adaptive alleles are present. If deleterious and adaptive alleles are considered together, the best strategy depends essentially on which of these types of alleles has the stronger influence on the change in total fitness. That should depend both on population size and on the hypotheses made concerning the three types of genes considered (initial frequencies, mutation rates, selective and dominance coefficients).

The variance in fitness over time due to deleterious genes increases as population size decreases, because homozygosity, as well as fixations



and accumulation of mildly deleterious genes, occur faster in small populations (Lynch et al. 1999). By contrast, fitness variance due to adaptive genes increases as population size increases, because higher genetic variability and larger effective population size allow faster adaptation (Gilpin and Soulé 1986; Lande 1988, 1995). The contribution of adaptive genes to total fitness variation becomes negligible in the case of a very small population with extremely weak allelic diversity. At the opposite, in the case of a large population, the contribution of deleterious mutations to total fitness variation is negligible.

#### *Optimal release strategy*

According to our results, the temporal organisation of the release may be chosen according to population size. It may be difficult, however, to determine a particular threshold above which the punctual release should be recommended, without specific data on the quantitative influences of adaptation and genetic deterioration on fitness. Empirical measures suggest that locally adaptive processes are rapid (Lenski et al. 1991; Dudley 1996) and have important consequences on fitness improvement, as suggested by the reduction of fitness induced by genetic introgression from distant provenance in plants (Keller et al. 2000) and the superiority of local populations in reintroductions (Griffith et al. 1989; Montalvo and Ellstrand 2000). However, the quantitative effect of adaptive mutations on fitness is difficult to assess, due the lack of estimates of their frequencies and selective effects, and their potentially high specificity. On the other hand, the role of genetic deterioration in species extinction has received much attention (Frankham et al. 2002). A clear pattern has emerged from numerous theoretical (Couvét 2002) and empirical (Richards 2000; Ebert et al. 2002; Saccheri and Brakefield 2002) studies, suggesting that immigration is extremely beneficial to small populations, owing to heterosis and genetic rescue.

Thus, in order to minimise the risk of making incorrect decision with respect to the rate of release, it should be recommended to establish a decision rule as a function of the expected impact of deleterious alleles, for which quantitative estimates of selective and dominance effects as well as mutation rates are available (see Drake et al. 1998). Therefore, from a genetic view-point, the

progressive release may be recommended for reintroduced populations with long-term effective size smaller than 50–100 (i.e., if the carrying capacity is smaller than few hundreds of individuals), for which genetic deterioration may pose a serious threat (Whitlock 2000). For larger populations, the increase of the genetic load may not be a major problem within conservation time frames and the punctual release, that maximises local adaptation, should be recommended.

For simplicity and generality, some properties inherent to real populations such as demographic and environmental stochasticities are not considered in our model. A measure of the relative fitness seems appropriate to evaluate the potential risk due to genetics in a particular demographic context. Since the viability of a population is dependent on many distinct complex mechanisms, it may be useful in a first step to study these processes separately in order to have the best understanding of them. However, confronting these mechanisms with each other is also necessary to evaluate the degree to which they interfere and their overall potential effect on viability. Further analysis including genetic, demographic and environmental factors as well as their interactions is therefore needed. Such analysis may be extremely useful when considering species restorations, a context in which hazard of failure is always present and success is mostly dependent on the knowledge and the understanding of the biological mechanisms involved in the growth and long-term bearing of populations.

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