

Transgenic Organisms

Risk Assessment of Deliberate Release

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Birkhäuser Verlag
Basel · Boston · Berlin

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Technologically modified genes in natural populations: some skeptical remarks on risk assessment from the view of population genetics

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Summary

Current theories of evolutionary and ecological genetics cannot be used for general statements on risk assessment of gene technology. In a simplifying model, the genes which are transferred from artificial populations are treated like mutations in the natural populations. It is shown how fast such mutants can become fixed depending on the transfer rate, the population size and the selection coefficient. However, our incomplete knowledge about living systems still does not allow reliable risk assessments because of our incomplete understanding of the underlying principles.

Introduction

It might be considered as symptomatic for the present status of our knowledge that no adequate theory exists which can be applied to risk assessment of technologically modified genes in natural populations. This is not astonishing if we take into consideration how complex the problem becomes if one starts to implement well-known non-linear gene interactions. Even if we knew about all possible effects and could use data to estimate their relative importance, this would not guarantee that reliable models could be built in the near future. This is not a pessimistic point of view; it can be inferred from the lack of extensive theory which connects genetics and ecology even for much simpler problems. For example, only recently has the influence of mutational load on the extinction of populations with density-regulated growth been studied (Lynch and Gabriel, 1990; Gabriel *et al.*, 1991). The astonishing and counter-intuitive results that result from synergistic interactions of genetic drift and population regulation (Gabriel *et al.*, 1993) show that there are still many unresolved complicated theoretical problems.

Theory cannot be expected to cover the whole complexity of reality. From simplified case studies we might get some hints and some qualitative understanding and perhaps the right order of magnitude of effects. In this paper I will discuss a single aspect and ask what are the consequences if a constant transfer of modified genes can be viewed as recurrent mutations. In reality, the transfer rate will not be constant, for example if there are only episodes of release

of modified genes. This might imply complicated dynamics which cannot be approximated by this simple model, but the model will still be useful for an initial rough estimation.

I will not ask general questions like: "Is there a difference between natural and artificial genes? How should these differences be defined? Can a gene be unnatural even if it is expressed by an organism? Is there a limit to man-made destruction of the environment above which further evolution is impossible? Is gene technology something other than more efficient breeding?" Such important questions need a lot of discussion, and the opinions will remain controversial, but I will express some general doubts on the possibility of risk assessment in natural systems.

The critical remarks in this paper should not be misinterpreted: I think that the research on molecular genetics is very important - but one should carefully debate whether and how academic research can be uncoupled from commercial interests and pressure. This might be an especially difficult task in the case of gene technology. Not only commercial interests but also the egoism resulting from the academic ambitions of researchers tend to underestimate or neglect the possible risks. I am not able to give an "objective" statement. My theoretical considerations are biased by thinking in terms of evolutionary ecology, but at least I am biased neither by commercial interests nor by the drive for a scientific career in molecular genetics.

Assumptions for a simplistic case

If new genes are not neutral, i.e. if they influence the reproductive success of their carriers, the fate of the new genes is determined not only by the population size *via* genetic drift but also by effects on fitness imposed by the transferred genes. The question is how to measure fitness. Even if one assumes that a gene influences only one trait and if one agrees on a fitness measure, it is not an easy task to estimate fitness in nature even if the determining fitness components are measurable under laboratory or field conditions. To get a complete picture of fitness one would have to accurately reconstruct the life history (Stearns, 1992) and to consider not only temporal and spatial changes in abiotic and biotic environmental components but also the complex relations between genotype and phenotype *via* phenotypic plasticity and reaction norms (Gabriel and Lynch, 1992).

We assume that fitness of individuals in a diploid population can be measured in a simple way and that the technologically modified gene can be treated as a mutant allele. We further assume that the "wild-type" has a fitness value of 1. We are not interested in the fitness advantage that the modified gene produces in the environment for which it is designed. We

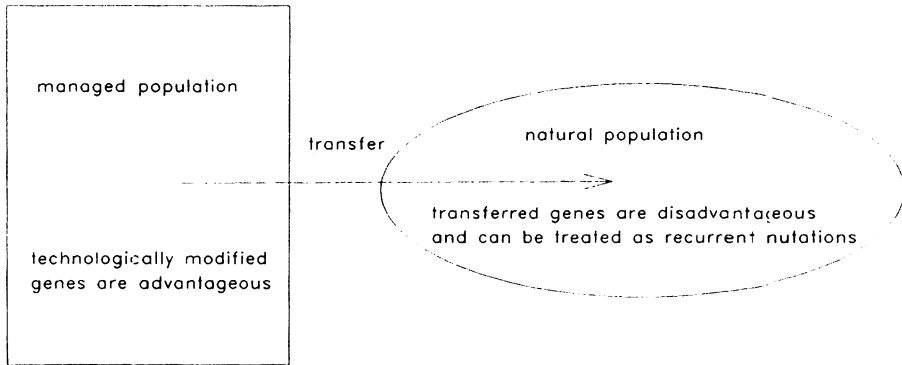


Figure 1. Simple case of transfer of genes into natural populations.

will study the case when such a transferred gene is disadvantageous in natural populations. Note that it is not important whether the gene is coming from the same species or from an unrelated one. (This might influence the transfer rate of genes but does not change the general problem.)

To keep the problem simple, we neglect all gene interaction and we make the unrealistic assumption that the fitness contribution of the transferred gene acts independently of all other genes which are present in the genome. We assume that the fitness of the "wild-type" is 1 and that a transferred gene changes fitness in heterozygotes to $1+h$ and to $1+s$ in the homozygotes (s and h are negative when the gene is disadvantageous. For the calculations we assume additivity of the genes, i.e. $h = s/2$).

If only a single copy of the transferred gene appears, the probability that this gene will become fixed in the population and the mean time until fixation can be calculated (see textbooks Ewens, 1979; Maynard Smith, 1989; Hartl and Clark, 1989). But we consider the case that a managed unnatural environment is a permanent source from which an unfavorable gene "escapes" and is transferred to natural populations (Figure 1). We will not specify the transfer rate, but as an initial rough estimate it is obvious that this rate increases linearly with the number of genes present in the managed population (or other technological products containing the genes). The transfer rate, therefore, increases with production area. This is important in quantifying risks emerging from long-term and large-scale industrial fields compared with small experiments on a short time-scale.

Transfer of genes viewed as recurrent mutations

The inflow of the genes is now treated like recurrent mutations occurring at a specified locus. It is worthwhile to remember one general result: In a finite population and under conditions of recurrent (irreversible) mutation the mutant allele, even when it is deleterious, will eventually become fixed. (Without limit on the time span this happens with a probability of 1).

If the population is large and if selection can act against the mutants (e.g., for s that is not too small and a low mutation rate), this time can be astronomically large and, therefore, biologically irrelevant. But we do not need unrealistic parameter values to produce substantial risks. The population size, for example, can be quite small. Note that one has to consider the "effective" population size, N_e , which is generally smaller - sometimes by orders of magnitudes - than the number of individuals in the population. The calculations are not trivial but there are approximations available under the assumption that s and h are small (Kimura 1985). Denoting the initial frequency of the mutant allele by p and the mutation rate by μ , the time until fixation $T(p)$ is approximately

$$T(p) = 4N_e \int_p^1 d\eta e^{B(\eta)} \eta^{-V} \int_0^\eta d\xi e^{B(\xi)} \xi^{V-1}/(1-\xi), \quad (1)$$

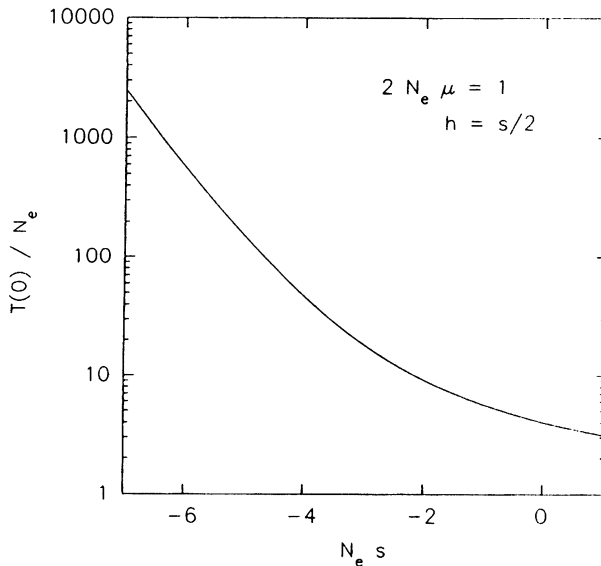


Figure 2. Time until fixation in units of N_e dependent on $N_e \cdot s$ under the assumption of $2N_e \cdot \mu = 1$.

where $B(x) = 2N_e \cdot s \cdot x^2 + 4N_e \cdot h \cdot x \cdot (1-x)$ and $V = 4N_e \cdot \mu$. The fitness modifications h and s are defined above. x , η , and ξ denote the frequency of the mutant allele and are used as integration variables. The integral can be solved numerically to get the mean time until fixation of a gene under recurrent mutations in finite populations. (We will consider populations which are free from mutations at the starting point and, therefore, calculate $T(0)$).

Figure 2 shows how time until fixation depends on the selection coefficient s . On the assumption that $2N_e \cdot \mu = 1$ (or equivalently with mutation rate $\mu = 1/2N_e$) this curve scales with the effective population size N_e if one plots the time until fixation in units of N_e depending on the product $N_e \cdot s$. (For $s = 0$, one gets the well-known result that the time until fixation is $4N_e$.)

In Figure 3 the mutation rate is kept constant ($\mu = 0.005$) and the dependence of the fixation time on s is shown for different population sizes ($N_e = 50, 100, 200, 400$). In our case, depending on the transfer mechanisms of the modified genes, the influence of population size will be more pronounced than that shown in the figure: a change in the population size would be associated with an opposite change in the mutation rate if the transfer rate remains constant.

To give an impression how variation in the mutation rate influences the fixation time, Figure 4 gives the dependence on mutation rate for the neutral case ($s=0$) for $N_e = 100$ and $N_e = 10^6$. The corresponding points $\mu = 1/2N_e$ (see Figure 2) are marked for easier comparison.

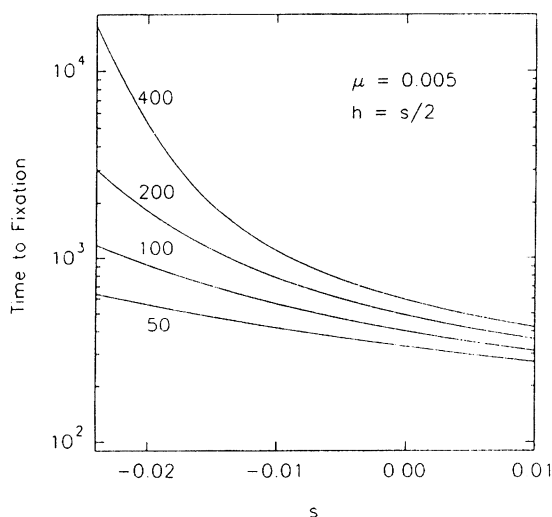


Figure 3. Time until fixation dependent on s for constant $\mu = 0.005$ and for $N_e = 50, 100, 200, 400$.

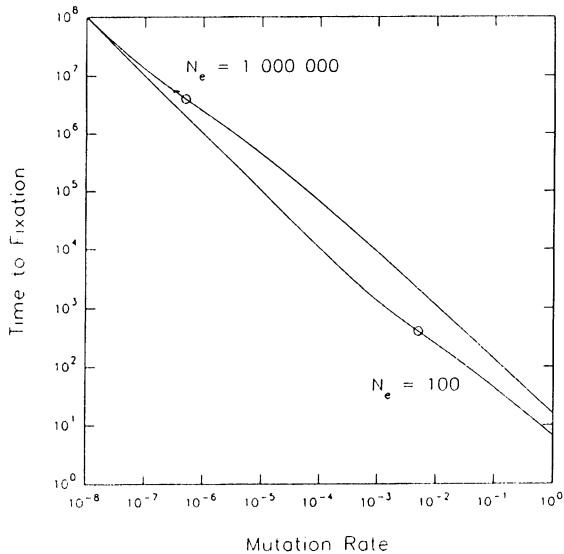


Figure 4. Time until fixation dependent on mutation rate for the neutral case ($s=0$) for $N_e = 100$ and $N_e = 10^6$. The points with $\mu = 1/2N_e$ are marked by circles.

We do not have any reliable estimates of transfer rates and selection coefficients. The calculations are done to demonstrate that - depending on the circumstances - there is a remarkable probability that natural populations will be inflated by bad genes which originate, for example from genetically modified organisms. It is important to point out that the danger emerges from the permanent flow of such genes. Under natural situations only some single mutant genes would appear, and they could easily be removed by natural selection.

Some basic doubts arising from the characteristics of the system

The above considerations are all based on oversimplifications e.g.; we neglected epistatic (synergism between loci) and pleiotropic (one gene influences several traits) effects. For quantitative traits epistatic interactions are the rule and not the exception (Wright, 1969; Barker, 1979). We did not consider that genes may have influence even if they are transferred into non-coding regions (Bernardi *et al.*, 1990). We did not discuss the dilemma that transferred genes might open new evolutionary pathways but can also break up useful constraints. We could continue to write down a long list of effects which are not considered on all levels starting, for example, with unresolved molecular mechanisms, problems regarding how different traits interact in fitness contribution, and feedback mechanisms at the population level between population dynamics and genetic composition. Even if there are many

unresolved problems as soon as we are able to describe the problem, we can try to find a solution. But risk assessment for technologically modified genes poses the following basic problem which arises from the characteristics of the systems involved.

Everybody who has worked on a large-scaled experiment in physics is most probably very skeptical about any risk assessment, because they might have experienced sudden unexpected errors which can occur despite very careful planning and checking. It is very easy to forget some possible events - or it is quite impossible to imagine all crazy possibilities in advance. Nevertheless, risk assessment might be possible to some degree in technical systems which are understood at least in principle - if one assumes that all possible errors and their combinations have been considered.

In living organisms, however, we are very far from a complete understanding of underlying principles and mechanisms. Organisms are not man-made machines and the functioning of organisms cannot be described and calculated like most physical and chemical processes. However, risk assessment is impossible without detailed understanding of all mechanisms. An honest scientist should be very careful before claiming that he can give a scientifically sound risk assessment. I do not deny that in special situations a statement like "according to our present knowledge this experiment is very unlikely to be risky" can be reasonable. This present knowledge, however, is far from being precise if compared with knowledge about technical systems. The name gene-"technology" does not guarantee that the problems imposed on the environment are understood and manageable like those in other technical disciplines.

Conclusions

We need further intensive research on molecular genetics - not only for obvious medical reasons, but because we have to admit that we are most often unable to predict the effects of molecular manipulations at the organismic and the population level. We have to clearly distinguish single and short-term experiments from long-term and large-scale industrial production. Viewing the transfer of genes as recurrent mutations might help us to obtain some qualitative understanding of risk determining factors.

Biologists should honestly state that the risk of technologically modified genes cannot be assessed with our present knowledge.

Acknowledgements

I thank Volker Loeschcke for his helpful comments and Nancy Zehrbach for improving the English.

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