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Invasion of transgenes from salmon or other genetically modified organisms into natural populations

Philip W. Hedrick

Abstract: In recent years, there has been widespread concern about the ecological and genetic effects of genetically modified organisms. In salmon and other fishes, transgenic growth hormone genes have been shown to have large effects on size and various traits related to fitness. In this paper, I have shown by using a deterministic model that if such a transgene has a male-mating advantage and a general viability disadvantage, then the conditions for its invasion in a natural population are very broad. More specifically, for 66.7% of the possible combinations of the possible mating and viability parameters, the transgene increases in frequency, and for 50% of the combinations, it goes to fixation. In addition, by this increase in the frequency of the transgene, the viability of the natural population is reduced, increasing the probability of extinction of the natural population. These findings provide independent confirmation of previous concerns about the inherent risks of transgenic organisms, especially for native salmon populations potentially affected by commercial salmon production using transgenic stocks.

Résumé : Depuis quelques années, les effets écologiques et génétiques des organismes génétiquement modifiés sont devenus l'objet de préoccupations universelles. Chez le saumon et d'autres poissons, les gènes transgéniques de l'hormone de croissance ont des effets importants sur la taille et sur d'autres caractéristiques reliées au fitness. L'utilisation d'un modèle déterministe a permis de démontrer que si un tel transgène procure un avantage aux mâles lors de l'accouplement et un désavantage pour la viabilité générale, les conditions qui permettent son insertion dans la population sont très larges. En particulier, dans 66,7% des combinaisons des valeurs possibles des variables reliées à l'accouplement et à la viabilité, la fréquence du transgène augmente et, dans 50% des combinaisons, il y a fixation du transgène. De plus, l'augmentation de la fréquence du transgène entraîne une réduction de la viabilité de la population naturelle et augmente la probabilité de son extinction. Ces résultats confirment de façon indépendante la pertinence des préoccupations concernant les risques inhérents aux organismes transgéniques, particulièrement pour les populations de saumons indigènes qui risquent d'être affectées par une production commerciale qui utilise des stocks transgéniques.

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Many genetically modified (GM) animals and plants utilize genes from other organisms, transgenes, to increase yield or change other economically important traits. Recently, Losey et al. (1999) showed that corn transformed with the bacterium *Bacillus thuringiensis* has detrimental effects on non-target monarch butterflies (however, see Wright et al. 2000), a report that raised great concern about the risks associated with GM organisms. In addition, if transgenes can spread by gene flow and selective advantage from GM or

ganisms into natural populations of the same or related species, then issues about GM organisms are further amplified.

Fish of several species transgenic for growth hormone genes are much larger than non-transgenic fish, and the muscular and molecular bases of this size increase has been documented in coho salmon, *Oncorhynchus kisutch* (Hill et al. 2000). Because larger males have increased mating success (Howard et al. 1998), these transgenes, once introduced by gene flow, may invade (increase in frequency) in a natural population because of this selective mating advantage. Transgenes may also potentially increase male mating success by changing factors such as mating behavior, color, etc. (W. Muir, personal communication). However, if the transgene also reduces viability, it may result in the extinction of the natural population because of reduced population fitness (Muir and Howard 1999). In this paper, I show that the conditions for invasion of transgenes and a consequent reduction in fitness are broad, raising great concerns about the

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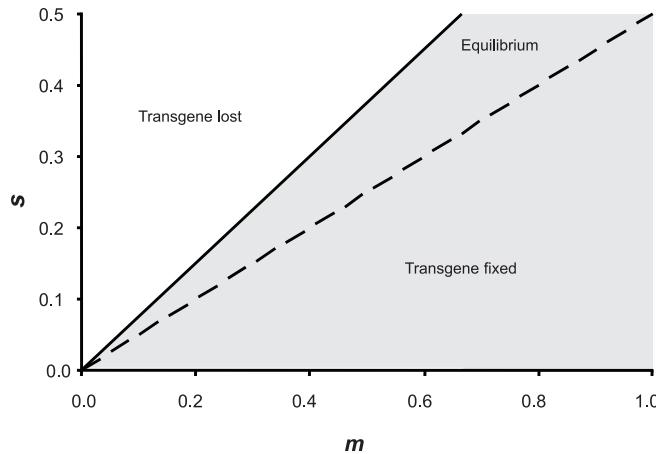
P.W. Hedrick. Department of Biology, Arizona State University, Tempe, AZ 85287, U.S.A. (e-mail: philip.hedrick@asu.edu).

Table 1. Proportions of the three types of progeny produced from the nine possible mating types.

Mating		Progeny		
Male	Female	A _t A _t	A _t A	AA
A _t A _t	A _t A _t	P ² /m̄	—	—
	A _t A	PH/2m̄	PH/2m̄	—
	AA	—	PQ/2m̄	—
A _t A	A _t A _t	PH/2m̄	PH/2m̄	—
	A _t A	P ² /4m̄	P ² /2m̄	P ² /4m̄
	AA	—	HQ/2m̄	HQ/2m̄
AA	A _t A _t	—	PQ(1-m)/m̄	—
	A _t A	—	HQ(1-m)/2m̄	HQ(1-m)/2m̄
	AA	—	—	Q ² (1-m)/m̄
		P' = p ² /m̄	H' = 2pq - mQp/m̄	Q' = q ² - mQp/m̄

Note: m is the mating disadvantage for wildtype homozygotes.

Fig. 1. The conditions for invasion of a transgene (shaded region), fixation of the transgene (to the right of broken line), and a stable equilibrium (between the solid and broken lines) as a function of the mating disadvantage (m) of the wildtype genotype lacking the transgene and the viability disadvantage (s) of the transgene genotypes. The mean fitness for the populations is reduced from 1 before the transgene is introduced to $1 - s$ after the transgene is fixed.



widespread use of GM organisms, particularly in production of salmon and other fishes.

Let us assume that A_t and A indicate the presence and absence of the transgene, with frequencies of p and q , respectively, and that genotypes A_tA_t , A_tA , and AA indicate homozygosity, hemizygosity, and the absence of the transgene (wildtype), respectively. Also assume that the relative male-mating successes of these genotypes are 1, 1, and $1 - m$, i.e., there is a mating disadvantage of m for the wildtype males, which do not have the transgene. For individuals of both sexes, assume that the relative viabilities of genotypes A_tA_t , A_tA , and AA are $1 - s$, $1 - s$, and 1, i.e., the two genotypes having the transgene have a viability disadvantage of s . Also assume that the frequencies of genotypes A_tA_t , A_tA , and AA are P , H , and Q , that all females are mated, and that the sum of the mating types is standardized

to unity. Therefore, the proportion of progeny genotypes from each of the nine possible mating types is as given in Table 1. The frequencies of the three genotypes in the progeny after mating and reproduction are (by summing the columns in Table 1)

$$P' = p^2/m̄$$

$$H' = (2pq - mQp)/m̄$$

$$Q' = (q^2 - mQp)/m̄$$

where $m̄ = 1 - mQ$. After differential viability, the genotypic frequencies become

$$P'' = P'(1 - s)/\bar{w}$$

$$H'' = H'(1 - s)/\bar{w}$$

$$Q'' = Q'/\bar{w}$$

where $\bar{w} = 1 - s(P' + H')$.

The conditions for invasion of the transgene can be determined by either iterating these equations or by assuming that $p'' = P'' + H''/2$ and by substitution from the expressions above to obtain

$$\frac{p''}{p} = \frac{1 - mQ(1 - s)/2}{1 - mQ - sp(1 + q - mQ)}$$

An increase in the frequency of the transgene occurs when $m > 2s/(1 + s)$, and if $m > 2s$, then the transgene invades and goes to fixation (Fig. 1). Overall, for 66.7% of the possible combinations of m and s , the transgene increases in frequency, and for 50% of the combinations, it goes to fixation. These straightforward relationships between the relative mating success and viability of organisms with and without the transgene simplify the design of future experiments to evaluate the potential invasion of a transgene and its impact on natural populations.

The fundamental theorem of Fisher (1958) predicts that the mean fitness of the population, \bar{w} , is expected to increase over time because of selection. However, in this situation, as the transgene invades as the result of selection for mating success favoring its increase, the mean fitness is reduced, not

increased. This occurs here because it is assumed that all females are mated, with or without the presence of the transgene, so that there is no influence of differences in mating success on mean fitness. In other words, the increase in the transgene does not increase the mean fitness through a mating advantage of the transgenic genotypes of the population. As the transgene increases in frequency, the mean fitness is reduced because the viability of individuals with the transgene is less than that of wildtype individuals. If the transgene becomes fixed, then $\bar{w} = 1 - s$ so that the relative fitness is reduced by an amount s . If this increased mortality results in a negative population growth rate, then the population will become extinct.

If $2s/(1 + s) < m < 2s$, then there is a stable equilibrium, as is also found for a similar antagonistic pleiotropy model (Hedrick 1999) with selection for female fecundity and viability. For the stable equilibrium for the transgene, the mean fitness is also reduced as a function of the equilibrium genotypic frequencies, i.e., $\bar{w} = 1 - s(P'_e + H'_e)$.

Several assumptions are inherent in these predictions. First, I have assumed that there is a negative pleiotropic effect of the transgene increasing mating success and reducing viability. This scenario is based on the emphasis of increasing body size in many transgenic experiments and the positive correlation of large body size and mating success (Muir and Howard 1999). Similar conclusions about the invasion of the transgene would occur if the associations are the opposite, i.e., the transgene reduces mating success and increases viability. On the other hand, if the transgene reduces both mating success and viability, then it would not invade, and if the transgene increases both mating success and viability, then it would always be predicted to invade. Second, for the population fitness to be reduced by the invasion of the transgene, it was assumed that all reproductive females are mated, regardless of the presence or absence of the transgene (also assumed by Muir and Howard 1999). In many fish populations of normal density, this assumption is probably nearly realized, but if the population size declines to low numbers, then this assumption may not be true.

The present model distills the parameters of mating advantage and viability disadvantage for transgenic organisms into two parameters. Muir and Howard (1999) examined the effect of these parameters on the time to extinction when the transgene invaded, but they did not provide the exact conditions for transgenic invasion. Muir and Howard (1999) used a deterministic, individual-explicit model to investigate the impact of a transgene invasion, which gave a detailed examination of the dynamical effects of the invasion on the transgene frequency and the population number. On the other hand, the simpler approach given here provides, in a traditional population genetic framework, a clearer understanding of the factors causing these initially counterintuitive results and the generality of the conclusions.

The description presented here simplifies the variation observed in life history, mating behavior, and other factors influencing the evolution and ecology of fishes. For example, details concerning the life history, population structure, and mating behavior of Pacific salmon (Groot and Margolis 1991) could be incorporated into a more realistic model. More specifically, the time spent in both freshwater and ma-

rine environments may be influenced by growth rate. Differences in time spent in these stages by transgenic and non-transgenic fish could potentially influence the dynamics and invasion of the transgene.

At this point, it is not clear whether fish transgenic for growth hormone genes will either have a mating advantage or a viability disadvantage. For example, Farrell et al. (1997) found that transgenic coho salmon are slower swimmers, possibly resulting in a viability disadvantage under natural conditions. On the other hand, Stevens et al. (1998) found that transgenic Atlantic salmon were more active than non-transgenic fish. Fitness differences between transgenic and non-transgenic fish will potentially depend on a number of factors, such as what species is receiving the transgene, what is the source species of the transgene, and the ecological context (Kapuscinski and Hallerman 1991; Reichhardt 2000).

However, as I have shown above, there are very broad conditions in which a transgene with a large mating advantage and a pleiotropic viability disadvantage may invade natural populations, reduce their fitness, and potentially cause their extinction. These findings should serve to alert researchers of the inherent risks of accidental releases of GM organisms into natural populations. In particular, consideration of the potentially very detrimental effects of accidental releases of transgenic fish and shellfish on natural populations should be of paramount concern in the commercial production of these transgenic organisms. For example, native salmon populations already under threat could potentially be further impacted by commercial salmon production using transgenic stocks.

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