

ORIGINAL ARTICLE

Male mating strategy and the introgression of a growth hormone transgene

Kata-Riina Valosaari, Sami Aikio and Veijo Kaitala

Integrative Ecology Unit, Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

Keywords

anadromous, Atlantic salmon, genotypic displacement, growth hormone transgene, introgression, invasion, sneaker.

Correspondence

Kata-Riina Valosaari, Integrative Ecology Unit, Department of Biological and Environmental Sciences, PO Box 65 (Viikinkaari 1), University of Helsinki, Helsinki, FIN-00014, Finland.
Tel.: +358 40 7400 346; fax: +358 9 7778 8445; e-mail: kata.valosaari@helsinki.fi

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Abstract

Escaped transgenic organisms (GMO's) may threaten the populations of their wild relatives if able to hybridize with each other. The introgression of a growth enhancement transgene into a wild Atlantic salmon population may be affected by the transgene's effects not only on fitness parameters, but also on mating behaviour. Large anadromous GMO males are most preferred in mating, but a transgene can also give the large sneakers a reproductive advantage over the smaller wild individuals. With a simulation model, we studied whether the increase in the proportion and mating success of sneakers in transgenic and hybrid genotypes could facilitate the introgression of a transgene into wild population after the release of GMOs. The model combines population dynamics and Mendelian inheritance of a transgenic trait. We found that the introgression of the transgene is strongly affected by the greater mating preference of large GMO males. Furthermore, the difference in reproductive success between the anadromous versus sneaker strategy defines how much GMO's have to be preferred to be able to invade. These results emphasize the importance of detailed knowledge of reproductive systems and the effect of a transgene on the phenotype and behaviour of GMOs when assessing the consequences of their release or escape to the wild.

Introduction

The research on growth enhanced transgenic fish for aquaculture benefits has been going on for two decades (Hulata 2001). It has also been recognized that fast growing fish may potentially threaten native populations if released or escaped to the wild (Muir and Howard 1999, 2001; Hedrick 2001a,b). Studies of the consequences of interbreeding between farmed escapees and wild fish have already demonstrated impoverishment of the gene pool of wild fish (outbreeding depression) by lowering the fitness of individuals (Hutchings 1991; Einum and Fleming 1997; McGinnity et al. 1997, 2003; Madeira et al. 2005; Roberge et al. 2007). This may lead to population decline or, in the worst case, local population extinction (Hutchings 1991; Fleming et al. 2000; Hindar et al. 2006). Another possible outcome of interbreeding between the native and introduced genotypes is the displacement of native geno-

type by the introduced one. Such genetic assimilation can take place if the introduced genotype is favoured in selection. Also a high immigration rate may result, with the introduced genotype displacing the native one (Huxel 1998).

The results of interbreeding between wild and farmed fish may not be directly applicable to the assessment of the consequences of interbreeding between wild and transgenic genotypes. For example, interbreeding between cultured (farmed) and native (wild) phenotypes of Atlantic salmon (*Salmo salar* Linnaeus, 1758) often produces intermediate hybrid phenotypes, because differences between them are usually a result of additive genetic differences (Einum and Fleming 1997; but see also Roberge et al. 2007). In contrast, a single copy of the 'all fish' growth hormone (GH) gene construct has already been shown to lead to rapid growth in, e.g. Atlantic salmon carrying the transgene (Du et al. 1992; Fletcher et al.

2004) suggesting that the transgene is dominant and thus the hybrid's phenotype is similar to transgenic homozygote.

Muir and Howard (1999, 2001) presented a model to define the outcome of a transgenic fish invasion into a natural population. They studied interbreeding between growth enhanced transgenic Japanese medaka fish and its wild conspecifics. The transgenic fish had an increased male mating success but reduced offspring viability. Muir and Howard (1999, 2001) showed that interbreeding of these two types could lead to fitness reduction of individuals in the natural population through outbreeding depression and in the worst case to local population extinction, although extinction may be due to the lack of density dependence in their model (Aikio et al. 2008). Muir and Howard referred to this observation as the Trojan gene effect.

Differences in mating behaviour may have an important role in determining the invasiveness and introgression of non-native traits into a wild population. Atlantic salmon males commonly have two alternative mating strategies, large anadromous and small mature parr males, which also differ in other life-history traits (Mills 1989). The decision over whether a juvenile male adopts the anadromous or mature parr strategy often depends on the size of an individual exceeding a threshold (Baum et al. 2004). The threshold may be determined by an individual's size or some other condition relative to other members of the population or the environment (Aubin-Horth and Dodson 2004). Larger juvenile males may mature early and stay in their natal river as a mature parr, while the smaller juvenile males migrate to sea to feed and grow (anadromous) before returning to reproduce in their natal river after a couple of years (reviewed by Fleming 1996). During mating, large anadromous males compete with each other to gain access to spawning females whereas small mature parr hide in the vicinity of the nest and sneak in to fertilize eggs once the anadromous males turn their back (Fleming 1996). Mature parr are thus called sneakers. Sneakers' reproductive success is also strongly dependent on the individual's size, as larger sneakers more often gain access to spawning females than smaller sneakers (Hutchings and Myers 1988; Thomaz et al. 1997; but see also Jones and Hutchings 2001). Despite the positive correlation between sneaker size and reproductive success, the relative success of sneakers decreases with increasing frequency of the sneaker strategy in the population (Thomaz et al. 1997).

It has been suggested that the sneaker strategy could act as a vector speeding introgression of the genes of farmed Atlantic salmon into natural populations (Garant et al. 2003; Hindar et al. 2006). This hypothesis has been presented because the sneakers of farm-reared and hybrid

Atlantic salmon have shown higher breeding and fertilization success than wild sneakers (Garant et al. 2003). In addition, the probability that the sneakers survive until the first breeding season may be higher than the survival of anadromous males. This is because sneakers stay in their natal river, mature early and may thus be able to escape the high mortality during the long-sea migration phase (Gross 1991).

Fast growth in transgenic salmonids has been shown to bring costs through pleiotropic effects, such as malformations, reduced predator avoidance, or a change in reproductive behaviour (reviewed in Devlin et al. 2004a; Sundström et al. 2004). However, growth enhanced transgenic Atlantic salmon males may be preferred in sexual selection as indicated by the observations that the primary mechanism in selection is contest competition where individuals with large size are preferred for mating (Fleming 1996; de Gaudemar et al. 2000).

We study how the risk of transgenic genotype invasion into a wild population can be affected by the proportion of offspring that employ the sneaker strategy and their relative mating success. Unlike the previous experimental (Garant et al. 2003) or modelling studies (Hindar et al. 2006) of the impact of alternative male mating tactics on the introgression of farmed traits into a natural population, we explicitly consider mating preference in our study. We built a simulation model to investigate the dynamics of wild, hybrid and GMO genotypes, defined by two alleles in one locus, after the introduction of the GMO genotype to a wild population. We investigated the various cases of sneakers presence in the offspring of different genotypes. These sneakers were allowed to vary in their mating successes.

Materials and methods

Model

The genotypes in the population are modelled by two alleles for growth in one locus, resulting in the wild (AA), hybrid (Aa) and transgenic (aa) genotypes. The hybrid genotype (heterozygote, Aa) in the model may also imply hemizygosity (genotype a0), where the A-allele corresponding to the transgenic a-allele is absent (Maclean and Laight 2000). Different scenarios of the dominance of the alleles affect on the individual's phenotype which are defined by the models parameters. The frequencies of different genotypes in the offspring resulting from different mating combinations follow Mendelian inheritance (Devlin et al. 2004b; Fletcher et al. 2004) (Table 1).

Mating success of male types

The frequency of mating between the three genotypes depends on their (i) frequencies in the population and

		♀		
		Wild, AA	Hybrid, Aa	GM, aa
♂	Wild, AA			
	Anadromous	AA	½ Aa + ½ AA	Aa
	Sneaker	AA	½ Aa + ½ AA	Aa
	Hybrid, Aa			
	Anadromous	½ Aa + ½ AA	¼ aa + ½ Aa + ¼ AA	½ aa + ½ Aa
	Sneaker	½ Aa + ½ AA	¼ aa + ½ Aa + ¼ AA	½ aa + ½ Aa
	GM, aa			
	Anadromous	Aa	½ aa + ½ Aa	aa
	Sneaker	Aa	½ aa + ½ Aa	aa

Table 1. Mating table for wild, hybrid and transgenic genotypes. There are two mating classes for males: anadromous males and sneakers.

(ii) the relative preference the males gain from females during mating. A central feature in our model is that males in each genotype can adopt either anadromous or sneaker mating strategy. Mating success between different mating types within each genotype is defined as the parameter q , $0 \leq q \leq 1$, which is the proportional mating success of the sneaker. Anadromous males mating success is thus $(1 - q)$. Changes in sneakers' mating success therefore affect the mating success of all anadromous males, although sneakers may not necessarily be present in each genotype.

Mating preference and genotype frequencies

The preference in mating of wild (WM), hybrid (HM) and transgenic (TM) males is expressed as the vector $\mathbf{p}_M^T = [(p_{WM}q + p_{WM}(1 - q), (p_{HM}q + p_{HM}(1 - q), 1 - (p_{WM}q + p_{WM}(1 - q)) - (p_{HM}q + p_{HM}(1 - q)))] = [p_{WM}, p_{HM}, 1 - p_{WM} - p_{HM}]$, where the uppercase T denotes a vector transpose. Preference in the mating from the opposite sex is a male trait (i.e. females may prefer differently males of different genotypes), and for a simplicity absent in wild (WF), hybrid (HF) and transgenic (TF) females (i.e. female genotype does not influence the preference of a male). Thus, the preference vector for the females is $\mathbf{p}_F^T = [1/3, 1/3, 1/3]$. The female preference \mathbf{p}_M is used as weighting factor by element wise multiplication (\circ) with the column vector $\mathbf{n}_M(t)$ for male densities, respectively, and scaled by the scalar product of the \mathbf{p}_M and $\mathbf{f}_M(t)$ vectors. The results of the vector $\mathbf{f}_M(t)$ for male mating frequencies at each genotype is then:

$$\mathbf{f}_M(t) = \frac{\mathbf{p}_M \circ \mathbf{n}_M(t)}{\mathbf{p}_M^T \mathbf{n}_M(t)}. \tag{1}$$

Male genotypes may thus differ in the amount females prefer them in mating but we assume that all female genotypes make the same preferences, i.e. the preference behaviour of females is independent of their genotypes.

The column vector $\mathbf{n}_F(t)$ for female densities gives the vector $\mathbf{f}_F(t)$ for female frequencies as a proportions of each genotype. The offspring are males and females in equal proportions.

Population renewal

The projection of population densities, i.e. sizes, from 1 year to the next is based on the 9-by-9 element transition matrix \mathbf{A} that is a sum of a reproduction matrix \mathbf{R} and survival matrix \mathbf{S} . The transition matrix \mathbf{A} represents both sexes and two life-history strategies for males (sneakers and anadromous) within the genotypes. Anadromous males experience 1-year delay in reproduction, but females do not delay their reproduction as sneakers accelerate it (i.e. age class for females). The six first columns of matrix \mathbf{R} are zeros, as males do not produce any offspring. The last three columns of \mathbf{R} represent female reproduction (Table 2), which is composed of each female's offspring genotype densities that are multiplied with fecundities F and frequencies of mating (genotype frequency \times preference in sexual selection). Fecundities are given as the number of viable offspring (surviving to the next breeding season) produced by each genotype. Some proportion P of male offspring adopts the sneaker strategy, while $1 - P$ adopts the anadromous strategy. Proportions of sneakers (P_{WPM} , P_{HPM} and P_{TPM}) may differ between genotypes. The density independent survival rates of each phenotype for the next breeding season compose a 9-by-9 element diagonal matrix (\mathbf{S}).

Population density is represented as the vector $\mathbf{n}(t) = [\mathbf{n}_M(t), \mathbf{n}_F(t)]^T$. Population dynamics follows the discrete time Beverton–Holt model (Beverton and Holt 1957):

$$\mathbf{n}(t + 1) = \frac{\mathbf{A}\mathbf{n}(t)}{1 + \mathbf{b}^T \mathbf{n}(t)}, \tag{2}$$

where the contribution to density dependence is proportional to each different genotypes' per capita biomass

Table 2. The three last columns of the 9-by-9 element reproduction matrix (R) of the offspring produced by each genotype according to their frequency (φ) in the population.

Offspring	Reproduction		
	Wild ♂	Hybrid ♀	Transgenic ♀
Wild ♂ _P	$F_W \frac{1}{2} P_{WPM} (f_{WPM} + f_{WAM} + \frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM})$	$F_H \frac{1}{2} P_{WPM} (\frac{1}{2} f_{WPM} + \frac{1}{2} f_{WAM} + \frac{1}{4} f_{HPM} + \frac{1}{4} f_{HAM})$	0
Wild ♂ _A	$F_W \frac{1}{2} (1 - P_{WPM}) (f_{WPM} + f_{WAM} + \frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM})$	$F_H \frac{1}{2} (1 - P_{WPM}) (\frac{1}{2} f_{WPM} + \frac{1}{2} f_{WAM} + \frac{1}{4} f_{HPM} + \frac{1}{4} f_{HAM})$	0
Hybrid ♂ _P	$F_W \frac{1}{2} P_{HPM} (\frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM} + f_{TPM} + f_{TAM})$	$F_H \frac{1}{2} P_{HPM} (\frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM} + \frac{1}{2} f_{TPM} + \frac{1}{2} f_{TAM})$	$F_G \frac{1}{2} P_{HPM} (f_{WPM} + f_{WAM} + \frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM})$
Hybrid ♂ _A	$F_W \frac{1}{2} (1 - P_{HPM}) (\frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM} + f_{TPM} + f_{TAM})$	$F_H \frac{1}{2} (1 - P_{HPM}) (\frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM} + \frac{1}{2} f_{TPM} + \frac{1}{2} f_{TAM})$	$F_G \frac{1}{2} (1 - P_{HPM}) (f_{WPM} + f_{WAM} + \frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM})$
Transgenic ♂ _P	0	$F_H \frac{1}{2} P_{TPM} (\frac{1}{4} f_{HPM} + \frac{1}{4} f_{HAM} + \frac{1}{2} f_{TPM} + \frac{1}{2} f_{TAM})$	$F_G \frac{1}{2} P_{TPM} (\frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM} + f_{TPM} + f_{TAM})$
Transgenic ♂ _A	0	$F_H \frac{1}{2} (1 - P_{TPM}) (\frac{1}{4} f_{HPM} + \frac{1}{4} f_{HAM} + \frac{1}{2} f_{TPM} + \frac{1}{2} f_{TAM})$	0
Wild ♀	$F_W \frac{1}{2} (f_{WPM} + f_{WAM} + \frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM})$	$F_H \frac{1}{2} (\frac{1}{2} f_{WPM} + \frac{1}{2} f_{WAM} + \frac{1}{4} f_{HPM} + \frac{1}{4} f_{HAM})$	$F_G \frac{1}{2} (f_{WPM} + f_{WAM} + \frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM})$
Hybrid ♀	$F_W \frac{1}{2} (\frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM} + f_{TPM} + f_{TAM})$	$F_H \frac{1}{2} (\frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM} + \frac{1}{2} f_{TPM} + \frac{1}{2} f_{TAM})$	$F_G \frac{1}{2} (\frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM} + f_{TPM} + f_{TAM})$
Transgenic ♀	0	$F_H \frac{1}{2} (\frac{1}{4} f_{HPM} + \frac{1}{4} f_{HAM} + \frac{1}{2} f_{TPM} + \frac{1}{2} f_{TAM})$	$F_G \frac{1}{2} (\frac{1}{2} f_{HPM} + \frac{1}{2} f_{HAM} + f_{TPM} + f_{TAM})$

♂, female; ♂_A, anadromous male; ♂_P, sneaker; P, proportion of sneakers in male offspring with subscript WPM, wild sneaker; WAM, wild anadromous male; HPM, hybrid sneaker; HAM, hybrid anadromous male; TPM, transgenic sneaker; or TAM, transgenic anadromous male to denote the genotype and the mating strategy of the male; F, fecundity with subscript W, H or G to denote wild, hybrid or transgenic genotype of the reproducing female.

$\mathbf{b}^T = [b_{WPM}, b_{WAM}, b_{HPM}, b_{HAM}, b_{TPM}, b_{TAM}, b_{WF}, b_{HF}, b_{TF}]$, i.e. larger individuals contribute more to density dependence than smaller ones. Matrix (S) expressing the survival probabilities is multiplied element-wise with the population density vector $\mathbf{n}(t) = [\mathbf{n}_M(t), \mathbf{n}_F(t)]^T$.

Analysis

We initiated the model system by simulating the dynamics of the wild genotype until it reached its equilibrium density. This initial phase was followed by an invasion attempt, where the transgenic genotype was introduced to the system in a range of initial densities selected between the extinction threshold level (10^{-6}) and the wild population's equilibrium density. We simulated the system for 1500 time steps to see the long-term outcome of the attempted invasion. We investigated a range of scenarios where genotypes and phenotypes differ in fecundity, survival, contribution to the density dependence, proportion of sneakers in male offspring and the female preference in mating.

Sneaker proportion and mating success

First, we analysed how the proportion and mating success of sneakers in the offspring affected the invasion of the transgene into the wild population. We made the analysis with sneakers present in only one genotype at a time. Genotypes were identical to each other with respect to other life-history characteristics (see parameterization in Table 3, genotypes identical). The invasion success of the transgenic genotype is as analysed in a parameter space of sneakers' mating success and sneakers proportion. The response variable is the proportion of hybrid and transgenic genotypes at the end of simulation.

Mating preference and sneakers' mating success

We investigated how changes in each genotype's mating preference, proportions and mating successes of sneakers affect the invasion of a transgene into the wild population. The effects of the changes were investigated within two cases, referred to as recessive and dominant, respectively, even though genotypes do not differ from each other in other life-history traits (for the parameterization of the model, see Table 3). In the recessive case, the wild and the hybrid genotypes had equal preference in mating, which differed from the preference for the transgenic. This implies that the wild and the hybrid genotypes each gain 50% of matings and the transgenic genotype will not gain matings at all. In the dominant case, the hybrid and the transgenic genotypes are equally preferred, meaning that when their preference in mating is zero, neither one is able to mate and the wild genotype gets all the matings. In the recessive case, only the transgenic genotype may have sneakers in

Table 3. Parameter values used in simulations when (1) genotypes are identical, (2) the transgene has additive effect, (3) the heterozygote experiences heterosis, (4) the transgene is recessive and (5 and 6) the transgene is dominant.

Parameter	Wild			Hybrid			Transgenic		
	δ_P	δ_A	♀	δ_P	δ_A	♀	δ_P	δ_A	♀
Contribution to density dependence (b , biomass)									
1. Genotypes identical	0.05	0.1	0.1	0.05	0.1	0.1	0.05	0.1	0.1
2. Transgene additive	0.05	0.1	0.1	0.07	0.2	0.2	0.09	0.3	0.3
3. Heterosis	0.05	0.1	0.1	0.09	0.3	0.3	0.05	0.1	0.1
4. Transgene recessive	0.05	0.1	0.1	0.05	0.1	0.1	0.09	0.3	0.3
5. Transgene dominant (1)	0.05	0.1	0.1	0.09	0.3	0.3	0.09	0.3	0.3
6. Transgene dominant (2)	0.05	0.1	0.1	0.09	0.3	0.3	0.09	0.3	0.3
Survival (S)									
1. Genotypes identical	0.100	0.050	0.050	0.100	0.050	0.050	0.100	0.050	0.050
2. Transgene additive	0.100	0.050	0.050	0.050	0.010	0.010	0.010	0.005	0.005
3. Heterosis	0.100	0.050	0.050	0.200	0.100	0.100	0.100	0.050	0.050
4. Transgene recessive	0.100	0.050	0.050	0.100	0.050	0.050	0.010	0.005	0.005
5. Transgene dominant (1)	0.100	0.050	0.050	0.010	0.005	0.005	0.010	0.005	0.005
6. Transgene dominant (2)	0.100	0.050	0.050	0.010	0.005	0.005	0.010	0.005	0.005
Fecundity (F)									
1. Genotypes identical			100			100			100
2. Transgene additive			100			75			50
3. Heterosis			100			200			100
4. Transgene recessive			100			100			50
5. Transgene dominant (1)			100			50			50
6. Transgene dominant (2)			100			50			50
Preference in sexual selection (<i>p</i>)									
1. Genotypes identical	0.333 <i>q</i>	0.333(1 - <i>q</i>)	0.333	0.333 <i>q</i>	0.333(1 - <i>q</i>)	0.333	0.333 <i>q</i>	0.333(1 - <i>q</i>)	0.333
2. Transgene additive	0.150 <i>q</i>	0.150(1 - <i>q</i>)	0.333	0.350 <i>q</i>	0.350(1 - <i>q</i>)	0.333	0.500 <i>q</i>	0.500(1 - <i>q</i>)	0.333
3. Heterosis	0.250 <i>q</i>	0.250(1 - <i>q</i>)	0.333	0.500 <i>q</i>	0.500(1 - <i>q</i>)	0.333	0.250 <i>q</i>	0.250(1 - <i>q</i>)	0.333
4. Transgene recessive	0.250 <i>q</i>	0.250(1 - <i>q</i>)	0.333	0.250 <i>q</i>	0.250(1 - <i>q</i>)	0.333	0.500 <i>q</i>	0.500(1 - <i>q</i>)	0.333
5. Transgene dominant (1)	0.150 <i>q</i>	0.150(1 - <i>q</i>)	0.333	0.425 <i>q</i>	0.425(1 - <i>q</i>)	0.333	0.425 <i>q</i>	0.425(1 - <i>q</i>)	0.333
6. Transgene dominant (2)	0.150 <i>q</i>	0.150(1 - <i>q</i>)	0.333	0.425 <i>q</i>	0.425(1 - <i>q</i>)	0.333	0.425 <i>q</i>	0.425(1 - <i>q</i>)	0.333
Proportion of sneakers (<i>P</i>)									
1. Genotypes identical	0.4 or varied 0–1			0.4 or varied 0–1			0.4 or varied 0–1		
2. Transgene additive	0.0			0.2 or varied 0–1			0.4 or varied 0–1		
3. Heterosis	0.0			0.4 or varied 0–1			0.0		
4. Transgene recessive	0.0			0.0			0.4 or varied 0–1		
5. Transgene dominant (1)	0.0			0.4 or varied 0–1			0.4 or varied 0–1		
6. Transgene dominant (2)	0.1 or varied 0–1			0.4 or varied 0–1			0.4 or varied 0–1		

δ_P , sneaker; δ_A , anadromous male; ♀, female.

Parameter *q* weights the mating success of sneakers in relation to anadromous males in genotypes.

offspring and in the dominant case, hybrid and transgenic may have sneakers in offspring. Sneakers proportions in the hybrid and/or transgenic genotypes were $P_{HPM} = P_{TPM} = 0.4$ within the simulations.

Sneakers' mating success in six selected cases

To investigate the importance of sneakers' mating success to transgene introgression into natural population, we analysed six possible cases where hybridization of wild and transgenic genotypes has different outcomes: (1) all genotypes are identical, (2) the transgene is additive, (i.e. heterozygote is intermediate of homozygotes), (3) the heterozygote has advantage over the two homozygotes,

aka. heterosis, (4) the transgene is recessive (i.e. heterozygote is like wild genotype), or (5 and 6) the transgene is dominant (heterozygote is like GMO genotype). We simulated two cases for the dominant transgene; in the first simulation, the wild genotype did not have sneaker males and in the second simulation the proportion of sneakers in the wild genotype was 0.1. Parameter values used in the six case studies are listed in Table 3. Parameter values were selected to illustrate different types of transgene inheritance and effect in the individuals that express the transgene. They do not represent any particular species or population, because actual data to parameterize transgenic salmon life-history characteristics

and behaviour in natural environments are lacking (Hallerman et al. 2007).

Results

Sneaker proportion and mating success

When sneakers were produced in wild or hybrid genotypes, their mating success determined if invasion was possible into a natural population. Hybrid and transgenic genotypes were present in the population, coexisting with the wild type, or the transgenic genotype replaced the wild type completely (Fig. 1). There was a clear threshold in sneakers' mating success at $q = 0.5$ that allowed invasion (Fig. 1A,B). Successful invasion did not affect the equilibrium population density in any of the cases studied (Fig. 1A–C).

When the mating success of wild sneakers was lower than anadromous males, i.e. $q < 0.5$, the wild genotype

was displaced by the transgenic genotype (Fig. 1.A1). If the mating success of wild sneakers was better than that of the anadromous males, invasion was not possible and wild was the only genotype present in the population (Fig. 1.A2).

Invasion was not possible if the mating success of hybrid sneakers was lower than the mating success of anadromous males (Fig. 1.B1). When sneakers were more successful in mating, all genotypes were present in the population, with the hybrid being the most common (Fig.1.B2).

Invasion success of the transgene into a natural population was determined with both the proportion of sneakers (P) and their mating success (q) when sneaking was an alternative mating tactic in the transgenic genotype (Fig. 1C). If the proportion of transgenic sneakers was higher than $P_{TPM} = 0.60$, their mating success had to be slightly higher than anadromous males ($q > 0.5$) to enable

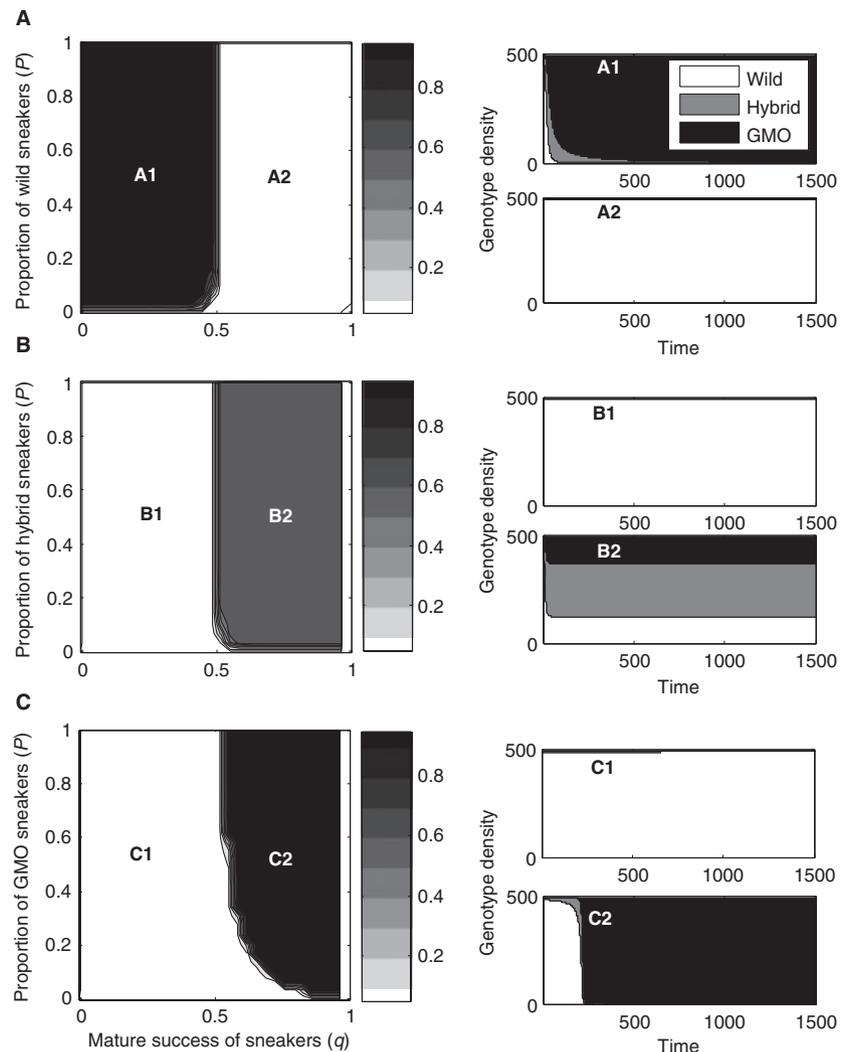


Figure 1 The effect of the proportion of sneakers P in the offspring of the three genotypes and the mating success q on the outcome of invasion. The shade of grey represents the proportion of hybrid and transgenic genotype in the population ranging from 0 (white) to 1 (black). Sneaker proportion P is varied separately within each genotype, (A) wild, (B) hybrid and (C) transgenic, while other genotypes did not produce sneakers. Genotypes are identical in other life-history traits. Time series show the genotype composition of the population when (A1–C1) the proportion of sneakers P is 0.5 and sneakers' mating success q is 0.25 and (A2–C2) sneaker proportion P is 0.5 and mating success q is 0.75 in relation to anadromous males.

invasion. With lower ($P_{TPM} < 0.5$) proportions of transgenic sneakers, the mating success of sneakers had to increase with decreasing sneaker proportion to allow invasion.

When the mating success of sneakers approaches $q = 1$, the wild genotype cannot initially grow to its carrying capacity because there are no sneakers present in the genotype. This leads to wild genotype extinction before attempted invasion and accounts for the strip of white parameter space at the right hand side of panels B and C in Fig. 1.

Mating preference and sneakers' mating success

When the transgene was recessive and all male offspring chose the anadromous strategy in the population, invasion of the transgene was possible if the transgenic genotype had at least $p_{TM} = 0.35$ preference in mating (meaning that wild and hybrid were both preferred with $p_{WM} = p_{HM} = 0.325$) (Fig. 2A). When the proportion of sneakers in the transgenic genotype's offspring was $P_{TPM} = 0.4$, sneakers' mating success influenced the required preference of the transgenic genotype in mating to enable invasion: with low transgenic sneakers' mating

success ($q = 0.10$), preference of $p_{TM} = 0.45$ was required for invasion (Fig. 2B) but when sneakers' mating success was high ($q = 0.70$), a preference of $p_{TM} = 0.25$ was sufficient (Fig. 2C).

When the transgene was dominant and all males anadromous, invasion of the transgenic genotype succeeded if the transgenic and hybrid genotypes were preferred with $p_{HM} + p_{TM} = 0.50$ (meaning that hybrid and transgenic genotypes were both preferred with $p_{HM} = p_{TM} = 0.25$) (Fig. 2D). When the proportion of sneakers in hybrid and transgenic genotypes, offspring was $P_{HPM} = P_{TPM} = 0.4$, low mating success of sneakers inhibited invasion if $p_{HM} + p_{TM}$ was less than approximately 0.60 (Fig. 2E) but high mating success enabled invasion when their proportion of mating preference surpassed approximately $p_{HM} + p_{TM} = 0.40$ (Fig. 2F).

Sneakers' mating success in six cases

Investigation of six case studies showed that the change in sneakers' mating success had different effect on the invasion success of the transgene depending on the effect the transgene has on the heterozygote or the transgenic homozygote (Table 3, Fig. 3). When all genotypes were

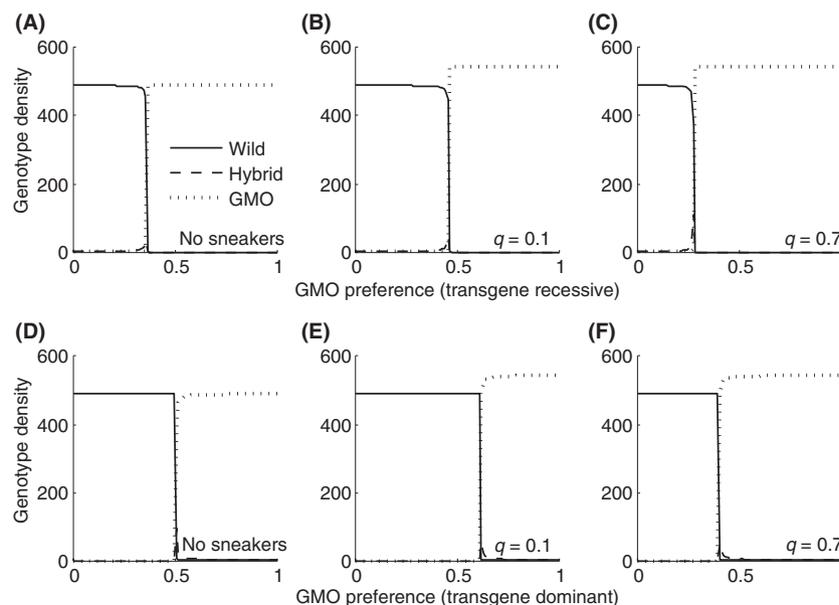
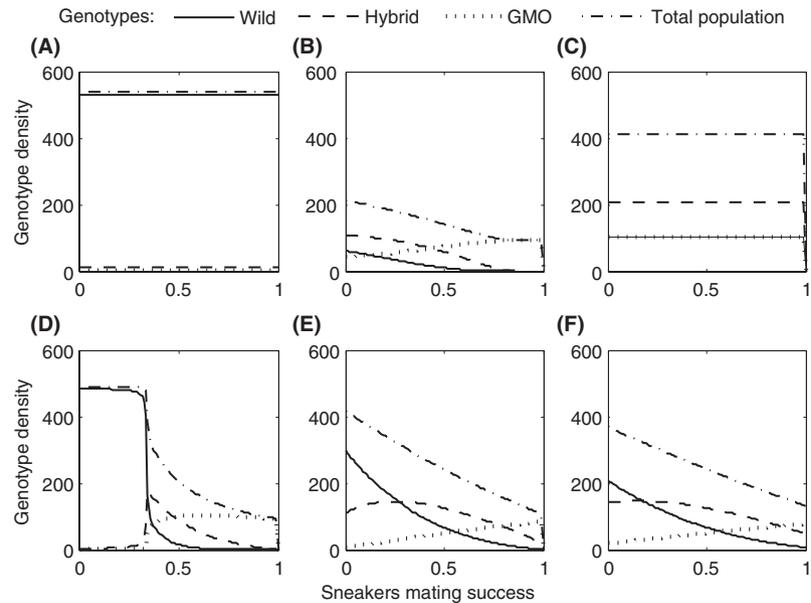


Figure 2 The effects of (i) the preference in mating p , (ii) the proportion of sneakers P in the offspring and (iii) sneakers' mating success q on the genotype densities in the population. The transgene is either recessive (hybrid and wild genotypes are preferred similarly in mating, panels A–C) or dominant (hybrid and transgenic genotypes are preferred similarly in mating, panels E–F). Genotypes are identical in other life-history traits. The cases with recessive transgene are: (A) no sneakers present in the population, (B) the proportion of sneakers in transgenic genotype $P_{TPM} = 0.4$ and the sneakers' mating success $q = 0.1$, (C) the proportion of sneakers in transgenic genotype $P_{TPM} = 0.4$ and the sneakers' mating success $q = 0.7$. The cases with dominant transgene are: (D) no sneakers present in the population, (E) the proportion of sneakers in hybrid and transgenic genotype $P_{HPM} = P_{TPM} = 0.4$ and the sneakers' mating success $q = 0.1$, (F) the proportion of sneakers in hybrid and transgenic genotype $P_{HPM} = P_{TPM} = 0.4$ and sneakers' mating success $q = 0.7$.

Figure 3 Variation in sneakers' mating success in relation to anadromous males affecting the genotype densities shown in six cases: (A) genotypes are identical ($P_{WPM} = P_{HPM} = P_{TPM} = 0.4$), (B) transgene has additive effect on hybrid, i.e. heterozygote is intermediate form of the homozygotes ($P_{WPM} = 0$, $P_{HPM} = 0.2$, $P_{TPM} = 0.4$), (C) heterozygote advantage aka. heterosis ($P_{WPM} = P_{TPM} = 0$, $P_{HPM} = 0.4$), (D) transgene is recessive ($P_{WPM} = P_{HPM} = 0$, $P_{TPM} = 0.4$), (E) transgene is dominant ($P_{WPM} = 0$, $P_{HPM} = P_{TPM} = 0.4$), and transgene is dominant ($P_{WPM} = 0.1$, $P_{HPM} = P_{TPM} = 0.4$). Parameter values for life-history traits are listed in Table 3.



identical, sneakers' mating success did not have an effect on invasion success or population density (Fig. 3A).

When the transgene had an additive effect on the heterozygote, i.e. hybrid was an intermediate form of the two homozygotes, population density declined with increasing sneakers' mating success (Fig. 3B). Also, the genotype composition of the population changed. Both wild and hybrid genotype densities decreased with increasing sneakers' mating success. Wild and hybrid went extinct when sneakers' mating success reached approximately $q = 0.75$. Transgenic genotype density increased when the others decreased. Total population density decreased with increasing sneakers' mating success from approximately 200 individuals to 150 individuals.

In the case of heterosis, sneakers' mating success did not affect the genotype composition or population density (Fig. 3C). All genotypes were present in the population, the hybrid being the most abundant.

Changes in sneakers' mating success had the largest impact on genotype frequencies and population density when the transgene was recessive (Fig. 3D). When sneakers' mating success was lower than approximately $q = 0.30$, only the wild genotype persisted. Population density collapsed and the wild genotype went extinct if sneakers' mating success increased above $q = 0.50$. Hybrid genotype density increased in the population when sneakers' mating success increased to $q = 0.30$, but with higher sneakers' mating success it decrease again and went to extinction when sneakers' mating success reached $q = 1$. Transgenic genotype density was the highest when the wild population collapsed and it stayed almost constant while sneakers' mating success increased from $q = 0.30$ to

$q = 1$. The total population density decreased with increasing sneakers' mating success from 500 individuals to <200 individuals.

In the first case when the transgene was dominant (panel E), the wild was the most common genotype in the population if sneakers' mating success was lower than $q = 0.25$ (Fig. 3E). In the second case, when also the wild genotype had sneaker males, the wild genotype decreased more rapidly (Fig. 3F). Wild genotype density thus decreased and transgenic genotype increased with increasing sneakers' mating success with both cases. Hybrid genotype density was relatively constant when sneakers' mating success was lower than $q = 0.50$, but decreased slowly when sneakers' mating success increased to higher values. Total population density decreased monotonously from 300 to 150 individuals with increasing sneakers' mating success and the total population density was lower with the case where also wild genotype had sneaker males when sneakers' mating success was low.

Discussion

Our modelling approach to investigate how alternative male mating strategies in Atlantic salmon affects the introgression possibilities of a transgene into natural population has shown that: (i) the introgression is more affected by sneakers' mating success than their proportion of the offspring; (ii) the introgression probability is mostly affected by the invading genotype's preference in mating but sneakers' mating success relative to anadromous males determines how much the genotype has to be preferred in order to invade; and (iii) invasion of a

transgenic fish with lowered fitness did not result in population extinction, but it led to population density decrease through outbreeding depression and also to genotypic displacement of the native genotype. These results emphasize that different kinds of behavioural differences within species, such as alternative mating strategies, have to be taken into account when assessing the possible hazards that transgenic organisms may cause if they invade natural populations.

Sneaker proportion and mating success

In the wild, the frequency of sneakers may vary greatly among Atlantic salmon populations, ranging from only few per cent of male juveniles adopting sneaker tactic to populations where all male juveniles mature at parr size (Fleming 1998; Hutchings and Jones 1998). Mating success has been shown to be both frequency- and status dependent, and thus, neither one of the strategies, sneaker or anadromous, may be evolutionary stable (Fleming 1998). Estimates of sneakers' mating success vary widely between different populations, from 23% up to 89% (Hutchings and Myers 1988; Morán et al. 1996; Thomaz et al. 1997) and also between different genotypes (Garant et al. 2003).

In our model structure, the proportion of sneakers could be varied separately in different genotypes. This is justified by the size-dependency of the choice of mating strategy and the fact that a sneaker's weight relative to its potential competitors is a better cue governing maturation than its absolute weight (Baum et al. 2004). However, initiation of maturation is dependent on growth rate/body size/lipid levels already approximately 1 year prior to maturation (Saunders et al. 1982; Silverstein and Shimma 1994; Silverstein et al. 1997, 1998). The adoption of the sneaker mating strategy by male juveniles in the model is thus status-dependent, meaning that only the largest juveniles can mature at parr size and benefit from it (Fleming 1998). However, the opposite may be true for some populations: Marschall et al. (1998) reviewed cases where, for example, in Scotland the fastest growing parr are those that migrate to the sea, whereas in Canadian populations they mature as parr size. In addition to genotype, the size of the juvenile is also determined by the size of the egg it hatched from, the timing of hatching, water flow and, nutritional conditions in the river, as well as population size (Gross 1991).

Sneakers' mating success, instead, affects anadromous males' mating success in all genotypes, such that when sneakers' mating success in some genotypes increases, anadromous mating success decreases regardless of the genotype. We found that the sneakers' mating success was a more important determinant for invasion than their

proportion in the genotypes. When sneakers have a higher mating success than that of anadromous males, the genotype that expresses sneaker strategy increases and the mating success of obligate anadromous genotypes is reduced. An individual sneaker's probability of fertilizing the eggs decreases with increasing sneaker number (Thomaz et al. 1997). Also, environmental factors have a strong effect on the mating success of the sneakers. Sneakers are not able to compete directly with anadromous males in mating, and their probability of fertilizing the eggs is also dependent on the presence of suitable hiding places in mating grounds where they can sneak to the females' nest during spawning (Myers and Hutchings 1987). Different measures of fish status other than size may therefore be needed to apply the model to other species and environments.

Sneakers' mating success and genotype preference in mating

It has been shown that the escaped farmed salmon anadromous males usually have lower mating success than that of wild salmon (Fleming 1996; Fleming et al. 2000; Weir et al. 2004). Our results showed that the mating preference of different genotypes forms an invasion threshold: transgenic fish cannot invade and the transgene (either recessive or dominant) cannot introgress into the wild population when transgenic males are less preferred in mating than wild males, when genotypes are otherwise identical. The lower mating preference means that transgenic males' spawning success is lower than wild males', in relation to their frequency in the population. This leads to lower fitness of transgenic males in comparison to wild males in our model, where fitness is defined as the likelihood of mating between members of the same genotype (Aikio et al. 2008). However, spawning success of the anadromous offspring of escaped farmed salmon has not been studied in detail (Hindar et al. 2006) and this is also true for growth enhanced transgenic salmon. We found that the invasion of transgenic fish succeeds if growth enhanced males (the transgenic homozygote when the transgene is recessive and both the transgenic homozygote and the hybrid when the transgene is dominant) are more preferred in mating than wild males and the invasion leads to transgenic genotypes displacing the wild genotype, which is an expected result.

The required mating preference (or spawning success) for growth enhanced genotypes to invade might change when the alternative mating strategies of the salmon are taken into account. Previous studies have suggested that sneakers could act as a vector facilitating the introgression of farmed genes into natural Atlantic salmon populations (Garant et al. 2003). The suggestion was based on the

results that farmed and hybrid sneakers have better fertilization success compared to wild sneakers, but the performance of farmed anadromous males was poor in reproduction. Our finding, that when transgenic or hybrid and transgenic sneakers' mating success is higher than anadromous males, even low mating preference of those genotypes enables invasion, is thus in line with Garant et al.'s (2003) results. We assumed that wild male juveniles adopt the anadromous tactic due to their smaller size in comparison to transgenic genotypes. However, lower mating success of sneakers in relation to anadromous males reduces the introgression potential of the transgene into the natural population, even when transgenics are preferred in mating. Low mating success of sneakers requires a higher preference of the transgenic and hybrid genotypes in mating to invade. These results suggest that sneakers' mating success partly determines invasion risk of transgene into natural population. Therefore, the sneaker mating strategy adopted by growth enhanced genotypes may either speed up or slow down the introgression of the transgene depending on its relative mating success. In nature, the shorter development time of sneakers compared to anadromous males may give them an extra advantage in mating, as they may be able to escape the higher mortality associated with the sea migration phase (Gross 1991).

Case studies

When investigating the outcome of transgene invasion with varying sneakers' mating success, the most interesting result was the case of a dominant transgene, because it has been shown that a single copy of GH transgene leads to rapidly growing phenotype in Atlantic salmon (Fletcher et al. 2004). This is the major reason why the outcome of hybridization of wild and transgenic individuals may differ from the hybridization between wild and farmed individuals. Interbreeding between farmed and wild Atlantic salmon usually results in an intermediate phenotype (Einum and Fleming 1997; but see also Roberge et al. 2007) which is comparable to our case where the transgene has an additive effect in the hybrid genotype.

The invasion of the growth enhanced transgenic salmon to the natural population led to population decline (outbreeding depression) in three of our six study cases through the alternative male mating strategy: the increase in sneakers' mating success decreased the total population size when the transgene was additive, recessive or dominant. The increase in sneakers' mating success increases the fitness of those genotypes in which male juveniles may adopt the sneakers' mating strategy and they may increase in density in comparison to those genotypes that

have only anadromous males. This means that if hybrid and/or transgenic genotypes were the only genotypes able to produce juveniles that may mature as parr, the increase in sneakers' mating success will increase their fitness in relation to the wild genotype. However, individuals expressing the transgene have also lower survival and fecundity despite their offspring maturing earlier and being preferred in mating due to larger size, which leads to a decline in population density. It is only when all genotypes have an equal number of sneakers, or the hybrid is heterotic, that the variation in sneakers' mating success does not lead to genotype displacement. However, when the hybrid is heterotic, the population size decreases in comparison to the case of identical genotypes.

When investigating different cases of transgene inheritance, the males expressing the transgene (both anadromous and sneaker) were considered to be preferred in mating despite their reduced survival and fecundity. This reflects the fact that larger males are usually better competitors gaining access to mate with females in Atlantic salmon (Myers and Hutchings 1987). There was no female preference among genotypes. This is a simplification of the model as large females are usually more fecund (able to produce viable eggs) than smaller ones (Fleming 1998). However, this may not be the case with farmed (Hindar et al. 2006) or with transgenic females. Fecundity of transgenic females may be lowered due to lower survival of smaller eggs, which is a consequence of larger female size (Hallermann et al. 2007).

Even though invasion of the transgene did not lead to local extinctions (a result opposite to Muir and Howard's 1999, 2001 findings), it did result in genotypic displacements of the wild genotype by transgenic and/or hybrid genotypes with increasing sneakers' mating success when the transgene was additive, recessive or dominant. When the hybrids are fertile and at least as fit as their parent genotypes, a possible threat of hybridization is the displacement of the other or both hybridizing genotypes from the population, resulting in a loss of genetic diversity in nature (Wolf et al. 2001). These results emphasize the importance of detailed knowledge of the reproductive systems of species in which a transgene is introduced. Understanding the effect of a transgene on phenotype and also on behaviour is essential when assessing the possible consequences of the accidental escape or intentional release of transgenic organisms in the wild.

The Atlantic salmon is not currently considered as a threatened species [classification in IUCN (2007) red list as a species of lower risk and least concern (LR/lc)], but many specific local populations are known where outbreeding depression could result in the loss of local adaptations (Kellogg 1999; Verspoor et al. 2005). From the conservation point of view, displacement of native

genotypes by artificial ones would be as serious a result as local population extinction. The use of growth enhanced salmon in aquaculture may thus be an appreciable conservation issue, which should be taken into account in decision making.

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