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ORIGINAL PAPER

Standing genetic variation and compensatory evolution in transgenic organisms: a growth-enhanced salmon simulation

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Abstract Genetically modified strains usually are generated within defined genetic backgrounds to minimize variation for the engineered characteristic in order to facilitate basic research investigations or for commercial application. However, interactions between transgenes and genetic background have been documented in both model and commercial agricultural species, indicating that allelic variation at transgene-modifying loci are not uncommon in genomes. Engineered organisms that have the potential to allow entry of transgenes into natural populations may cause changes to ecosystems via the interaction of their specific phenotypes with ecosystem components and services. A transgene introgressing through natural populations is likely to encounter a range of natural genetic variation (among individuals or sub-populations) that could result in changes in phenotype, concomitant with effects on fitness and ecosystem consequences that differ from that seen in the progenitor transgenic strain. In the present study, using a growth hormone transgenic salmon example, we have modeled selection of modifier loci (single and multiple) in the presence of a transgene and have found that accounting for genetic background can significantly affect the persistence of transgenes in populations, potentially reducing or reversing a "Trojan gene" effect. Influences from altered life history characteristics (e.g., developmental timing, age of maturation) and compensatory demographic/ecosystem controls (e.g., density dependence) also were found to have a strong influence on transgene effects. Further, with the presence of a transgene in a population, genetic backgrounds were found to shift in non-transgenic individuals as well, an effect expected to direct phenotypes away from naturally selected optima. The present model has revealed the importance of understanding effects of selection for background genetics on the evolution of phenotypes in populations harbouring transgenes.

Keywords Transgenic · Background genetics · Compensatory evolution · Modifier · Fitness · Risk · Salmon · Growth · GH · Trojan gene

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Introduction

Genetically engineered fish have now been in development for more than 25 years (Zhu et al. 1985) for use as basic research models and for commercial

application. A major focus of research has been the production of transgenic fish that are growth enhanced compared to founder strains, which in some cases (e.g., mud loach and coho salmon) has resulted in very large increases in body size of up to 35- to 37-fold at specific ages (Devlin et al. 1994; Du et al. 1992; Martinez et al. 1996; Nam et al. 2001; Pitkanen et al. 1999; Rahman et al. 1998; Venugopal et al. 2004). Some of these strains have been proposed for use in aquaculture, which has prompted concern regarding potential ecological consequences should such fish accidentally enter natural ecosystems (Reichhardt 2000; Stokstad 2002). Large changes in growth phenotype in transgenic fish are associated with many underlying cellular and organismal physiological and behavioural changes, some of which have the potential to alter survival and reproductive success (fitness) of such fish should they enter natural ecosystems. Understanding the phenotypic effects of the transgene, as well as how these changes may influence interactions with ecosystem components, is necessary when assessing risk to natural populations. Ecological effects on ecosystems of transgenic strains are anticipated to depend on many factors: (1) the probability of entry to the ecosystem (by escape from confinement, or by purposeful introduction), (2) the survival and reproductive success (fitness) of the escaped individuals as well as their naturally-reared offspring, and (3) the consequences arising from use of ecosystem resources or genetic disruptions affecting the adaptability of populations (Devlin and Donaldson 1992; Devlin et al. 2006; Kapuscinski et al. 2007a, b; Kapuscinski and Hallerman 1990; Tiedje et al. 1989). Relative phenotypic and fitness differences between transgenic fish, non-transgenic conspecifics, and other ecosystem members are strong determinants of the probability of initial persistence and subsequent spread of a transgene, and hence the magnitude of potential consequences. The present paper seeks to examine the theoretically evolution of fitness and consequences of transgenic strains in nature arising from interaction between the transgene and other modifier loci in the genome.

Empirical assessments of engineered fish often are conducted by comparing the progenitor non-transgenic strain with a well-defined, often inbred, transgenic strain that reproducibly display uniform physiological and behavioural phenotypes. Since potential fitness effects and consequences of a transgenic strain will be highly dependant on its phenotypic differences from wild-type and/or other ecosystem members, understanding the potential for the transgenic phenotype to vary is critically important to allow accurate estimates of risk. Phenotypic variation in a transgenic strain can arise from several factors, including environmental effects, developmental plasticity, or epigenetics (Bessey et al. 2004; Devlin et al. 2004b; Devlin and Donaldson 1992; Hails and Morley 2005; Sundström et al. 2007; Weichman and Chaillet 1997). Variation also can arise from genetic changes, including alterations in transgene structure or from background allelic differences among individuals. In most natural fish populations, significant quantitative genetic variation exists which can influence the growth rate among individuals and strains (e.g. Dickerson et al. 2005; Gjedrem 2000; e.g. McClelland et al. 2005; Smoker et al. 1994; Tymchuk et al. 2006; Tymchuk and Devlin 2005). Such background genetic differences have the potential to interact with transgenes and influence phenotype, an effect that has been described in laboratory studies with other animal and plant systems (Alatalo et al. 2008; Eisen et al. 1993; Eisen et al. 1995; Engler et al. 1991; Linder 2001; Metz et al. 2006; Nadeau 2001; Nielsen et al. 1995; Parisi et al. 2003; Robertson et al. 2002; Siewerdt et al. 1998; Siewerdt et al. 2000; Suzuki et al. 2002; Weng et al. 1995). In mice, modifiers of GH transgenes have been observed and have been found to be responsive to artificial selection and to undirected natural selection (in the laboratory) that can strongly modify phenotype (Chaudhry et al. 2008; Siewerdt et al. 2000). Some of these transgene modifiers have been genetically mapped and have been found to act through the pathway of the gene of interest, or alternatively to affect epigenetic processes such as gene silencing by methylation (Dragani et al. 2000; Engler et al. 1991; Kantachuvesiri et al. 1999; Valenza-Schaerly et al. 2001). In transgenic fish, a transgene overexpressing growth hormone (GH) has been found to have very different effects depending on genetic background. For example, in coho salmon, the GH transgene was found to work synergistically with a domesticated genome to enhance growth, whereas in rainbow trout, a wild (slow-growing) strain was found to be stimulated much more by the transgene than was a domesticated strain previously selected for very rapid growth (Devlin et al. 2001).

Transcript profiling and endocrinology studies of GH transgenic and domesticated strains have shown that both influence gene expression patterns in similar ways relative to wild type (Devlin et al. 2009; Fleming et al. 2002; Raven et al. 2008; Tymchuk et al. 2009). Together, these observations reveal how a GH transgene can function differently among genetic backgrounds in vertebrates, and can have reduced influence on growth if previous traditional genetic selection of natural genetic variation has already exploited much of the potential opportunity to modify growth pathways and phenotype (Kitami and Nadeau 2002).

To facilitate assessments of potential ecological risks of transgenic fish, several fitness-based population models incorporating life history characteristics and ecological parameters have been developed. Modeling approaches have provided extremely valuable tools for examining the range of fitness parameter values that are expected to influence the persistence of a transgene in populations (Davis and Fulford 1999; Davis et al. 1999; Hedrick 2001; Maclean and Laight 2000; Muir and Howard 1999, 2001; Muir and Howard 2002). Several of these studies (Aikio et al. 2008; Davis and Fulford 1999; Maclean and Laight 2000) have identified the importance of initial frequency, repeat introductions and relative differences in fitness of transgenic and wild-type fish. Muir and Howard (1999, 2001, 2002) have further outlined major survival and reproductive fitness parameters anticipated to affect transgene persistence and population size. Their simulations also have shown that a transgene causing antagonistic pleiotropic effects (e.g., enhanced mating success, with reduced viability) could invade a population under specific conditions (Hedrick 2001), sweep into a population, and cause subsequent decline or extinction of the population, an outcome termed a Trojan gene effect (Muir and Howard 1999, 2001, 2002). These hypothetical scenarios advanced our thinking significantly regarding modeling risk of transgenic organisms, and recently Aikio et al. (2008) have shown further that density-dependence influences (which commonly act in fish populations) can overcome Trojan-gene effects and prevent population extinctions. Other modeling (Davis et al. 1999) has revealed that including stochasticity in survival and birthrate influences the movement of a transgene into populations (e.g., higher variance yields faster transgene introgression), and Valosaari et al. (2008) have modeled the effect of mating success and age of maturation (within an evolutionarily stable strategy) on transgene frequencies in populations.

Previous modeling efforts have defined transgenic organisms as genetically and phenotypically uniform, and have not incorporated effects of background genetic variation that could result in the evolution of altered phenotype. Given the evidence that transgene modifier loci exist among laboratory strains, it is reasonable to assume that in nature, a transgene is very likely to encounter a range of genetic variation different from that found within the engineered strain (Devlin and Donaldson 1992; Devlin et al. 2007; Kapuscinski et al. 2007a). Over generations, the average phenotype caused by the transgene could evolve as selection acts on background alleles to improve fitness of individuals and populations. Such changes could influence consequences to ecosystems from that predicted from assessments made in the laboratory using a single strain with a relatively inbred genetic background. To examine these influences and extend previous models, we have undertaken an exercise to explore the potential for background genetic variation to influence transgene persistence in, and effects upon, populations. The present study has focussed on GH-transgenic coho salmon (*Oncorhynchus kisutch*) (Devlin et al. 2004a), which have been used as a model for transgenic fish risk assessment research (Devlin et al. 2006; Sundström et al. 2007). The theoretical population model developed allows background genetics (2-locus, or polygenic) to partially or fully modify the survival and reproductive fitness of transgenic and non-transgenic individuals through multiple overlapping generations in simulated populations. Our results indicate that background genetics has the potential to play an important role in mediating the persistence of transgenes in populations, and that evolutionary shifts in these populations may affect non-transgenic conspecifics as well.

Methods

Modelling effects of fitness parameters and background genetics on persistence of GH transgenic salmon in populations

A deterministic, age-structured model was developed to explore the theoretical effects of an introduction of

GH-transgenic individuals into a wild salmonid population. The approach is similar to that previously developed by Muir and Howard (1999, 2001) except the present model focuses on salmonid life histories, incorporates potential effects of density-dependent juvenile survival, and includes effects of selection on background genetics (2-locus and polygenic) for multiple fitness parameters. For the 2-locus model, numbers of individuals and genotype frequencies over time were simulated assuming diploidy, with one modifier and one transgene locus (potentially 10 genotypes). One locus determines presence (transgene) or absence (wild type) of the transgene (A_T or A, respectively), with the transgene having a dominant phenotypic effect ($A_TA = A_TA_T$). The second locus can act to modify (positively or negatively) fitness parameter values both for transgenic and wild-type genotypes (alleles B or B_M; the form B_M hereafter referred to as the 'modifier'). The effects of the modifier were assumed to be additive, with B_MB_M causing the greatest compensatory epistasis. Initial frequency of the B_M polymorphism was arbitrarily assumed to be present in 10% of wild-type individuals.

Allowing for genotypic variability in female (j) fecundity (F_j), male (k) mating success(m_k), genotype (i)-specific survival through the first year of life ($s^j_{i,t}$), and assuming Mendelian segregation ($M^r_{i,j,k}$) under a given recombination rate r, expected numbers of offspring of genotype i within the first age-class ($N^j_{i,t}$) was modeled as

$$N^j_{i,t} = s^j_{i,t-1} \sum_{j=1}^{10} \sum_{k=1}^{10} \left(p_{k,t-1} F_j N^f_{j,t-1} M^r_{i,j,k} \right). \quad (1)$$

The probability of a male (l) mating ($p_{l,t}$) depended on male mating success (m_k) and male abundance ($N^m_{k,t}$) such that

$$p_{l,t} = m_l N^m_{l,t} \left(\sum_{k=1}^{10} m_k N^m_{k,t} \right)^{-1}. \quad (2)$$

Male mating success was expressed relative to wild-type mating success. Juvenile survival ($s^j_{i,t}$) in any year (t) depended on total egg deposition (E_t), and was modeled assuming that age-one recruitment followed the common Beverton-Holt form of recruitment relationship. Allowing for genotype-specific relative viability (V_i), a maximum wild-type survival rate (α), and a parameter (β) that represent density-dependent effects, juvenile survival was modeled as

$$s^j_{i,t} = \alpha V_i (1 + \beta E_t)^{-1} \quad (3)$$

Maximum survival rate of wild-type juveniles (α) was parameterized as

$$\alpha = \Omega \phi_e \quad \text{where } \phi_e = 0.5 F_w s^A \left(\varepsilon_w + (1 - \varepsilon_w) s^{J2} \right). \quad (4)$$

In this parameterization, Ω is the relative improvement in juvenile survival at low egg deposition levels from the juvenile survival expected under undisturbed conditions. φ_e is the expected lifetime egg production of a wild-type individual and depended on wild-type female fecundity (F_w), the proportion of the population smolting after 1 year in fresh water (ε_w) and the survival of smolts to return as adults the following year (s^A), was assumed constant within a particular scenario. The proportion of juveniles that did not smolt after 1 year ($1 - \varepsilon_w$) was subjected to an additional survival adjustment (S^{J2}) to account for additional fresh water losses. These individuals then survived at s^A to account for smolt-to-adult losses. The β parameter can be also represented in terms of Ω, φ_e, and maximum smolt production (R_0) such that

$$\beta = (\Omega - 1)(R_0 \phi_e)^{-1}. \quad (5)$$

This parameterization is useful because juvenile production can be expressed in terms of life history characteristics, maximum juvenile production, and the relative strength of compensatory response in juvenile survival. For the simulation presented, female fecundity was assumed to be 4,000 eggs, smolt-to-adult survival was assumed 20%, S^{J2} was assumed to be 50%, and the proportion of wild-type individuals smolting after 1 year was 10%. As a result, maximum egg to age-one survival, assuming weak compensation ($\Omega = 2$), was around 1%; with $\Omega = 5$, survival increased to 2.2%. Relative genotype-specific juvenile viability (V_i) was assumed constant across all densities and potentially reduced for transgenic individuals. Females and males were assumed to spend 1 or 2 winters in freshwater, depending on genotype, and one winter in the ocean. Adult returns of a particular genotype and sex in a given year ($N^{f\,\text{or}\,m}_{i,t}$) were modeled as

$$N_{i,t}^{f\text{ or }m} = \varepsilon_i 0.5 N_{i,t-1}^{J} s^{A} + (1-\varepsilon_i) 0.5 N_{i,t-2}^{J} s^{J2} s^{A}. \quad (6)$$

Juvenile sex ratio was assumed to be 50:50, and the proportion of juveniles smolting after 1 year (ε_i) was dependent on genotype.

For a number of simulations, the effects of the transgene were assumed to be influenced by multiple loci. In these polygenic scenarios, selection for favourable backgrounds was modeled as a progressive change in the transgenic phenotype toward the wild type. For the simulations presented in this paper it was assumed that juvenile viability, male mating advantage, and the proportion of juveniles smolting after 1 year had all changed relative to wild type. Equation 7 is an example of the calculation applied to viability:

$$V_{T,g+1} = V_{T,g} + \vartheta(V_w - V_{T,g}). \quad (7)$$

Here, $V_{T,g}$ is the transgenic viability in generation (g), V_W is the wild-type vulnerability, and ϑ is the proportion of the difference between wild-type and transgenic viability reverted each generation. Thus, transgenic individuals asymptotically approach the wild type in terms of juvenile viability, male mating advantage, and the proportion of juveniles smolting after 1 year.

Density-dependant controls affecting survival were also present in the model. Relative change in equilibrium population size can be calculated using Eq. 8 for scenarios that resulted in transgene fixation. Under scenarios where the transgene reached fixation, relative change in population size was described using Eq. 9, and Eq. 10 defined the lower bound of Ω.

$$\frac{N_{eq}^{T}}{N_{eq}^{W}} = \frac{(V_T \Omega \phi_e^T - \phi_e^W)(-\varepsilon_T - S^{J2} + S^{J2}\varepsilon_T)}{\phi_e^T(\Omega - 1)(-\varepsilon_T - S^{J2} + S^{J2}\varepsilon_W)} \quad (8)$$

$$\frac{N_{eq}^{T}}{N_{eq}^{W}} = \frac{V_T(-\varepsilon_T - S^{J2} + S^{J2}\varepsilon_T)}{(-\varepsilon_W - S^{J2} + S^{J2}\varepsilon_W)} \quad (9)$$

$$\Omega_c = \phi_e^W / V_T \phi_e^T \quad (10)$$

A number of assumptions and simplifications are inherent in the model in order to facilitate description of the background genetic effects. As in Muir and Howard (1999) and Hedrick (2001), we have assumed a negative pleiotropic effect of the transgene that increases mating advantage in males, and reduces viability over the first year in freshwater for both sexes. This scenario allows for more complex analysis of interacting fitness components than if transgenes possess simply elevated or reduced net fitness. All reproductive females were assumed to mate and to have equal fecundity. Rapid growth of transgenic salmon may allow precocious smolt transformation (Devlin et al. 1994, 2000; Saunders et al. 1998), and thus 90% of transgenic individuals were assumed to smolt after their first year compared to 10% for the wild type. It is important to note, however, that the true development of transgenic fish in nature is suspected to be very different (i.e. slower) from that observed under hatchery conditions (Sundström et al. 2007). The relative increase in egg to first-year survival (Ω) was assumed similar for all genotypes regardless of the viability reduction caused by the transgene. For all simulations, population abundance was initialized at equilibrium given a maximum production of wild-type juveniles by the end of their first year in freshwater ($R_0 = 2\text{x}10^5$). Introduction of transgenic individuals (A_TBA_TB) was simulated as a single event with an abundance of 0.1% of the equilibrium wild-type spawning population (N_0).

The model also allowed for varying the linkage relationship between the modifier locus and the transgene, from tight linkage (0% recombination) to distant linkage (50% recombination). However, there was little effect unless the modifier was tightly linked in cis (for example mimicking a structural change at the transgene locus). Thus, scenarios were modeled with modifiers in trans and unlinked (most modifiers are anticipated to in reality be in this state relative to the transgene).

Results

Potential effects of altered fitness parameters and life history traits on transgene frequency and population size in GH transgenic salmon

Base simulations with the model (without action of modifiers) yielded results similar to those previously described (Davis and Fulford 1999; Davis et al. 1999; Hedrick 2001; Maclean and Laight 2000; Muir and Howard 1999, 2001; Muir and Howard 2002). The model also effectively approximated salmon population dynamics, responding as expected to variation in survival, age of maturation, reproductive fitness

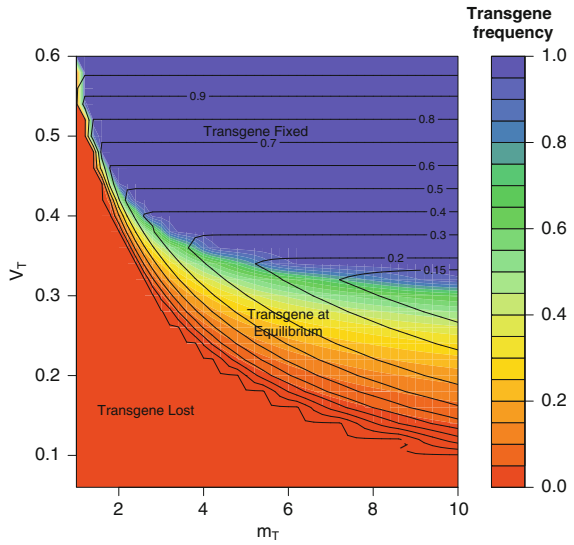

Fig. 1 Equilibrium spawning population relative to the pure wild-type equilibrium population size (N_{eq}^T/N_{eq}^W, *black contour lines*) and transgene frequency (A_T) (*colour shaded contours*) as a function of relative transgenic age-1 viability (V_T) and transgenic male mating advantage (m_T) assuming no effect of background genetics on the transgene. Viability and mating advantage were scaled relative to wild type. *Darkest blue shading* indicates fixation of the transgene while *dark orange* indicates the transgene is lost. 90% of transgenic juveniles were assumed to smolt after 1 year in this scenario, while only 10% of wild juveniles smolted after 1 year. Relative improvement in juvenile survival at low density was set low ($\Omega = 2$)

parameters, and life history variation. The scenario depicted in Fig. 1 shows that when fitness components with opposing effects (enhanced male mating success, coupled with reduced survival as first year juveniles and in the smolt to adult interval) are combined, the transgene is able to invade the population over a wide range of conditions, reaching either fixation or a stable equilibrium. The presence of the transgene also can reduce mean population fitness and result in lower equilibrium population size (N_{eq}^T) compared to a wild-type (N_{eq}^W) population ($N_{eq}^T/N_{eq}^W < 1$, i.e., a 'Trojan gene' effect). Relative change in population size (Eq. 8) depends on juvenile survival rate reduction for individuals possessing the transgene (V_T) as well as the strength of density-dependent compensatory changes in juvenile survival (Ω; Fig. 2a). Expected lifetime fecundity of GH-transgenic (ϕ_e^T) and wild-type (ϕ_e^W) individuals, survival during the second year of freshwater residence, and the proportion of individuals smolting after 1 year of freshwater residence (ε_W or ε_T), also can strongly influence changes in allele frequency and population size. It is important to note that in simulations where a high proportion of transgenic individuals matured at only 2 years of age and the juvenile survival reduction associated with the transgene was weaker (i.e., 80% of wild-type), equilibrium population size increased even when male mating advantage was only twofold (solid grey line Fig. 2a). When viability effects increased (i.e. 50% of wild type) at the same mating advantage, population size did not increase and smaller reductions in population size were predicted relative to the more extreme scenario ($V_T = 0.3$ and $m_T = 5$). Reduction in mean generation time of transgenic individuals caused transgene frequencies and relative equilibrium population size to increase, even when juvenile viability was reduced. Increased juvenile survival compensation (Ω) reduced the relative decline in population size. Under scenarios where the transgene reached fixation, relative change in population size approached an asymptote described by Eq. 9. Although compensatory changes in survival in response to reduced population density prevents population extinction under a 'Trojan gene' scenario, strong reductions in population size are still possible when density dependent effects on juvenile survival (Ω) are weak. Equation 10 defines the lower bound on Ω (Fig. 2b). Below these values of Ω, under a 'Trojan gene' scenario, population extinction occurred. It is important to note that as the viability of transgenic individuals declines, the critical bound on Ω increases geometrically.

Potential effects of background genetics on transgene effects

We now consider how modification of the fitness of transgenic individuals due to variation in background genetics may influence the outcome of transgene introductions into natural populations. In many cases, background genetics would differentially affect various fitness parameters in transgenic individuals, for example by ameliorating disease susceptibility effects (e.g., Jhingan et al. 2003), but not affecting mating advantage or age of maturity. Thus, the model allows modifier effects to be applied differentially to individual fitness parameters. The effects of background genetics were modeled initially with a single modifier

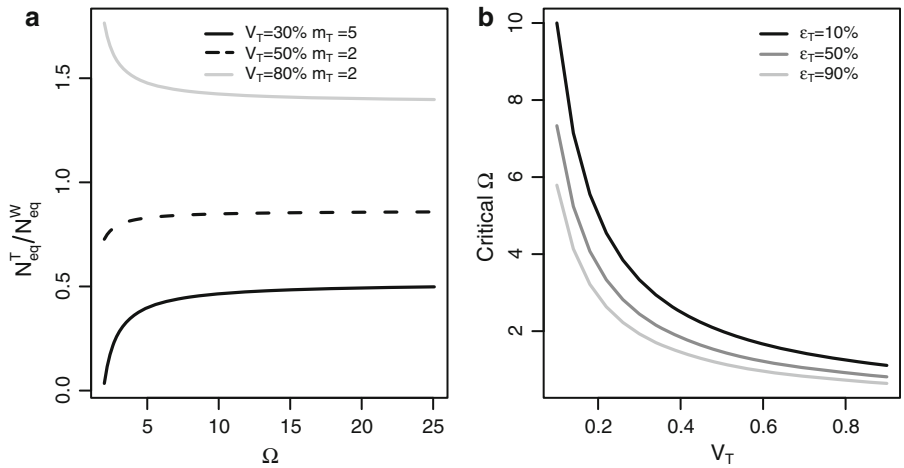

Fig. 2 Change in relative equilibrium population size as a function of the strength of compensatory change in juvenile survival (Ω) for a 'Trojan gene' scenario (Panel **a** *black solid line*) where transgenic viability was 30% ($V_T = 0.3$) of wild type and the male mating advantage was 5 times ($m_T = 5$). This is contrasted with less extreme scenarios where transgenic viability was 80% ($V_T = 0.8$) of wild type and the male mating advantage was 2 times ($m_T = 2$) (*solid gray line*) and where transgenic viability was 50% ($V_T = 0.5$) of wild type and the male mating advantage was 2 times ($m_T = 2$) (*broken black line*). Panel **b** displays how the critical compensation ratio (Ω) changes as a function of viability differences in transgenic individuals (V_T) and the proportion of transgenic individuals smolting after 1 year (ε_T). Below the critical compensation ratio, a Trojan gene scenario resulted in population extinction

locus present in the population that, in one allelic form (B_M), counters the effect of the transgene on multiple fitness parameters. For the example shown (Fig. 3), the relative juvenile viability, mating advantage, and maturity schedules of individuals possessing the transgene with two copies of the modifier (B_M) were assumed to be halfway between the wild-type and transgenic phenotypes. Individuals with only one copy of the modifier were assumed intermediate between transgenic individuals and those possessing two copies of the modifier. Given these compensatory epistatic effects of the modifier, the transgene reached fixation (blue area) over a broader range of scenarios (juvenile viability x mating advantage combinations), with the modifier reaching fixation (white hatched area) over a range of scenarios as well. Population levels with the modifier (Fig. 2) were depressed over much of the range examined, although the severity of impact was strongly reduced compared to scenarios lacking the modifier (Fig. 1). The range of scenarios under which the transgene was maintained at a stable equilibrium by frequency-dependent selection also was substantially compressed (compare the size of coloured zones between dark blue and dark red in Figs 1 and 3). Over a range of scenarios (white hatched area), transgenic individuals with the modifier gained a fitness advantage, transgene spread was enhanced, and the modifier became fixed. This region of modifier fixation exhibits transitions into zones of scenarios where transgenic individuals with and without the modifier are in a stable equilibrium, with the modifier being maintained in the population through frequency-dependent selection. At higher levels of viability, transgenic individuals without the modifier can have a greater fitness advantage and the modifier is lost from the population. As the modifier spreads in the population, improved viability of transgenic individuals due to the presence of the modifier can ameliorate population declines (compare Figs 4a and b). Although the severity of population impact is reduced under 'Trojan gene' scenarios, background variation that was neutral in the absence of the transgene experienced selection in the presence of the transgene. As a result, in the scenario presented, the modifier reached fixation (compare Figs 4d and e). If the modifier fully ameliorated the effects of the transgene (Fig. 4c), the fitness advantage of the 'modifier-transgene' construct was reduced. As a result, population recovery was less and the modifier did not reach fixation. If a heterozygote advantage for non-transgenic individuals

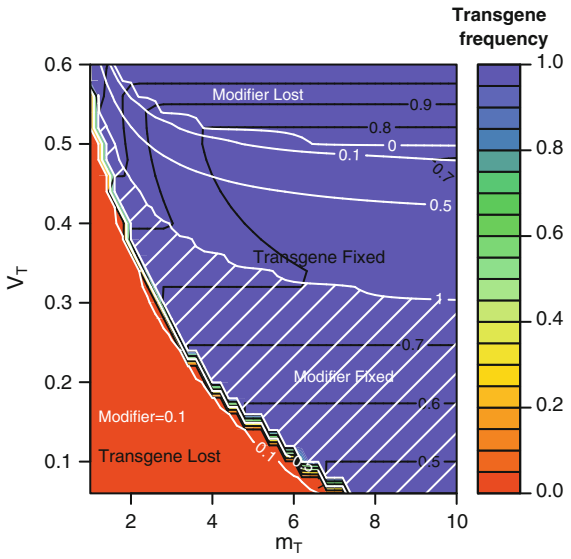

Fig. 3 Equilibrium relative spawning population size N_{eq}^T/N_{eq}^W (*black contour lines*), transgene frequency (A_T) (*colour shaded contours*) and modifier frequency (B_M) (*white contour lines and hatching*) as a function of relative transgenic juvenile viability (V_T) and transgenic male mating advantage (m_T). Viability and mating advantage are scaled relative to wild type. *Blue shading* indicates fixation of the transgene ($p(A_T) = 1$), while dark orange indicates that the transgene is lost ($p(A_T) = 0$). *Hatched white area* indicates fixation of the modifier allele ($p(B_M) = 1$). Modifier (B_M) frequency within the white area is at its initial frequency (0.1). Relative equilibrium spawning population size (N_{eq}^T/N_{eq}^W) is scaled relative to the equilibrium spawning population in the absence of the transgene. 90% of transgenic juveniles are assumed to smolt after 1 year in this scenario, while only 10% of wild-type juveniles smolt after 1 year. Relative improvement in juvenile survival at low density was set low ($\Omega = 2$)

existed at the modifier locus, variation can be maintained by balancing selection. However, in the presence of a transgene, alleles that are unfavourable in homozygous form for wild type were selected and caused a more rapid decline in wild-type numbers.

When the interaction of many genes was assumed to ameliorate the effects of the transgene (Eq. 7), the dynamics of the population were somewhat different. In these polygenic scenarios, if selection for favourable backgrounds was low (Fig. 4g), the transgene reached fixation and the reduction in population abundance due to 'Trojan gene' effects was reduced. On the other hand, if selection was rapid (Fig. 4h), and reversion of the transgenic phenotype occurred before the transgene reached fixation, the transgenic genotype becomes a stable polymorphism.

The simulation results presented so far have all assumed that a high proportion (90%) of transgenic individuals smolt after 1 year and mature in 2 years as is seen for GH transgenic coho salmon under laboratory conditions (Devlin et al. 2004b). The relative maturity schedules affect both the range of viability reductions and mating advantages over which the transgene introgressed as well as the equilibrium population size. When transgenic individuals are assumed to mature on a wild-type schedule (e.g. 3 years), a 'Trojan gene' effect was observed at lower mating advantages and at smaller viability reductions (Fig. 4i). When a high proportion of transgenics were assumed to mature at age 2 (90%) a 'Trojan gene' effect is not seen and the population equilibrium size is higher than with wild-type alone. Changing the maturity schedule of transgene fish effectively shifts the zone described in Fig. 1 upwards.

Discussion

The present modeling exercise, using a growth-enhanced coho salmon as a theoretical example, examined the importance of background genetic effects in modifying the outcome of transgene introductions into populations. The model allows examination of the effect of selection for background genetic alleles that partially or fully overcome negative fitness traits associated with a transgene. When genetic backgrounds ameliorate the effects of the transgene, it was found that the range of conditions over which the transgene can invade a population was increased, and that the transgene can be maintained in equilibrium by frequency-dependant selection under some conditions. Under circumstances where a transgene would otherwise be lost, novel genetic backgrounds can facilitate persistence of the transgene over a broader range of conditions if selection for favourable backgrounds occurs at a rate greater than the inherent loss rate for the transgene. Thus, modifier alleles causing fitness impairments in the wild-type that counter-select the transgene phenotypes are anticipated to increase in frequency in populations and influence ecological consequences arising from transgenic individuals.

Growth rate in fish is a continuous quantitative character that is polygenically influenced by (mainly) additive genetic variance (e.g., Gjedrem 2000;

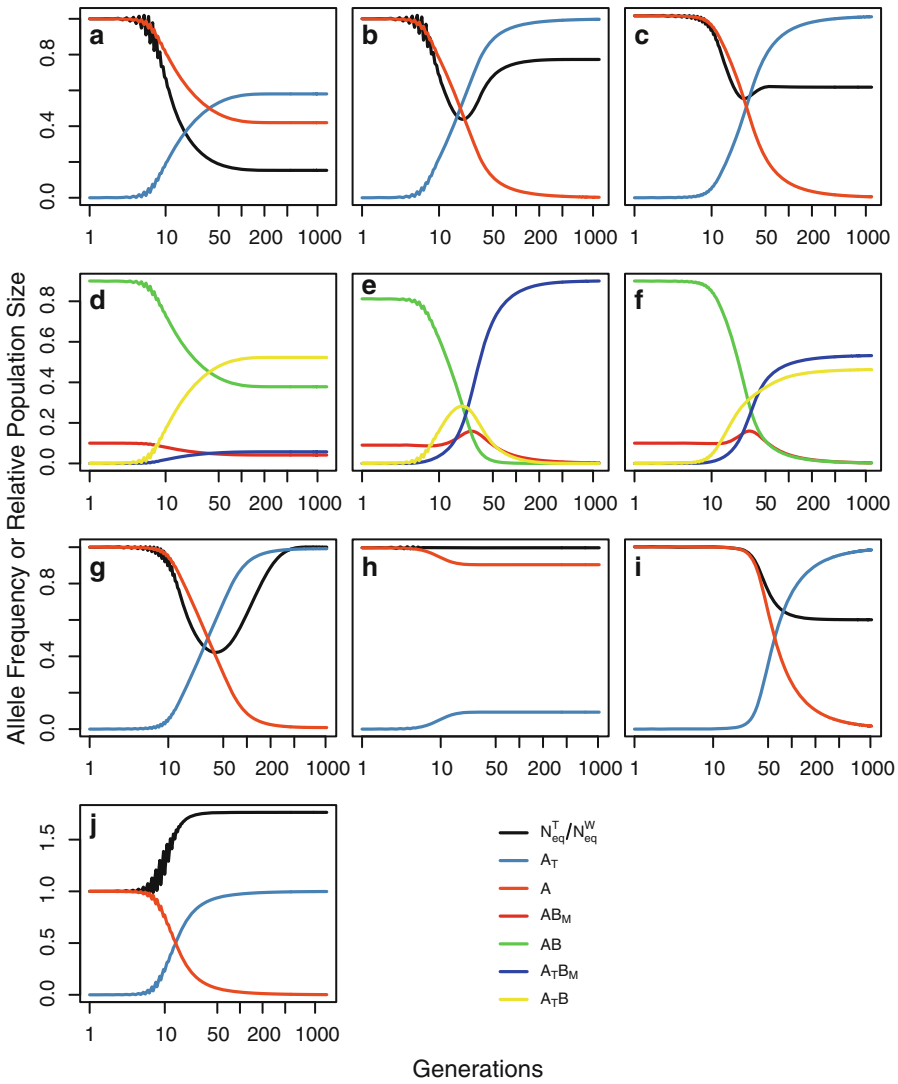

Fig. 4 Equilibrium population sizes and gene frequencies from 'Trojan gene' scenarios where unmodified transgene viability was set to 0.3 and transgenic mating advantage was set at 5. Relative improvement in juvenile survival at low density was set low ($\Omega = 2$). Panels **a** and **d** represent relative population and gene frequency changes in the absence of the modifier, and panels **b** and **e**, as well as **c** and **f**, represent these changes in the presence of the modifier. In panels **b** and **e**, the 'modifier-transgene' phenotype is half way between the wild-type and transgenic phenotypes, and in **c** and **e** the 'modifier-transgene' phenotype is equal to the wild type. Panels **g** and **h** are from polygenic scenarios where selection intensity was assumed to be low $\vartheta = 0.002$ (*d*) or high $\vartheta = 0.1$ (*h*). Panels **i** and **j** are provided for contrast. In panel **i**, transgenic viability and mating advantage are set to 0.8 and 2, respectively, but transgenic individuals smolt and mature at the same time as wild type. In panel **j**, transgenic viability and mating advantage are set to 0.8 and 2 respectively but transgenic individuals do smolt and mature 1 year earlier than wild type. In panels **a**–**c** and **g**–**j**, the *black line* indicates N^T_{eq}/N^W_{eq}, the *red line* is the frequency of wild-type genotype, and the *blue line* transgenic genotype. In panels **d**–**f**, changes of the genotypes are represented: AB_M the wild type with the modifier (*red line*) and AB without the modifier (*green line*). The *blue* and *yellow lines* are the frequencies of the transgene with (A_TB_M) and without (A_TB) the modifier, respectively. The initial modifier frequency was set to 0.1. 90% of transgenic juveniles were assumed to smolt after 1 year in all scenario scenarios but I, while only 10% of wild juveniles smolted after 1 year

McClelland et al. 2005; Tymchuk et al. 2006; Tymchuk and Devlin 2005) and is associated with reasonably high heritabilities and responses to selection (Dickerson et al. 2005; Gall and Huang 1988; Gjedrem 2000; Hershberger et al. 1990; Smoker et al. 1994). Wild fish have long been selected for optimal growth rate, but for many species, growth rates in nature are below that which is physiologically possible. For example, salmonids are capable of being strongly growth stimulated through environmental, nutritional, or hormonal manipulations (Ali et al. 2003; Donaldson et al. 1979), suggesting that less than maximal growth rates are adaptive in nature and arise from fitness tradeoffs with other components of Darwinian fitness, for example, involving traits pertaining to physiological requirements (such as disease resistance), or to minimize predation risk from foraging (Arendt 1997; Lima and Dill 1990). Indeed, elevation of growth rate away from wild type (e.g. in GH transgenic or domesticated salmon) can result in increased risk taking and reduced fitness via increased predation mortality (Abrahams and Pratt 2000; Abrahams and Sutterlin 1999; Biro et al. 2004; Sundström et al. 2004, 2005; Tymchuk et al. 2005; Tymchuk et al. 2006; Tymchuk et al. 2007). Thus, background genetic variation that reduces growth rate of transgenic fish back towards the natural fitness optimum probably would be selectively favoured in natural populations. Further, observed pleiotropic effects of GH transgenesis on morphology, physiology, and behaviour could have negative fitness consequences (e.g. Bessey et al. 2004; Deitch et al. 2006; e.g. Farrell et al. 1997; Leggatt et al. 2003; Ostenfeld et al. 1998; Stevens and Devlin 2000; Sundt-Hansen et al. 2007), and selection at modifier loci that compensate for these specific effects would be anticipated. For example, disease impairments in GH transgenic salmon (Jhingan et al. 2003) might be overcome by selection at immune function loci, and could do so without altering the enhanced growth rate. Thus, modifiers are expected to be prevalent in the genome and of multiple types, affecting growth generally or targeting specific pleiotropic phenotypes caused by the transgene.

Population size effects arising from transgene-modifier interactions were apparent in the present study in both the two-locus and polygenic simulations. The present salmon model has predicted Trojan gene effects on population size (e.g., as shown by Hedrick 2001; Muir and Howard 1999, 2001) under specific conditions, but these consequences could be ameliorated to a large degree (2 locus model) or completely (polygenic) by selection occurring at modifier loci. As such, transgene-countering loci would be anticipated to be selected for in nature, thereby restoring population level fitness. In an analogous system in nature (segregation distortion, SD), meiotic drive can cause chromosomes with SD alleles to preferentially segregate into populations, which, if sex linked, can skew sex ratios and cause population declines (Hamilton 1967). Thus, it is not surprising that extant populations containing SD alleles also possess modifiers that counter these effects (Jaenike 2001). Huang et al. (2007) modeled mosquito-control transgenes being driven into populations by a meiotic drive system, and have found that the presence of unlinked genetic modifiers can strongly influence the spread of the transgene. In the present model, in addition to population reduction effects, we also observed scenarios where genetic modifiers acted in concert with the transgene to cause population increase beyond those seen with wild type alone. This outcome might occur, for example, if fitness 'valleys' could be bridged to allow development of novel phenotypes unachievable by populations using extant natural allelic variation, or because of strong stabilizing selection.

In addition to genetic compensatory responses arising from modifier-transgene interactions, the present model also predicted that demographic controls can strongly dampen population effects associated with transgene-induced Trojan gene scenarios. Consistent with the findings of Aikio et al. (2008), density-dependent effects causing increased survival at lower population sizes are capable of minimizing negative fitness effects of transgenes. Reductions in population size can still ensue from antagonistic pleiotropic effects even in the presence of compensatory responses; however, for the scenario presented herein mimicking the salmon life history, very large increases in mating advantage of transgenic fish over wild type were required to induce strong depressions in population size. Further, none of the scenarios we examined predicted population extinctions, even when mating advantage was 10-fold greater than wild type (a biologically unrealistic value).

Shifts in life-history characteristics caused by growth enhancement may prove critical in influencing transgene effects in populations. For example,

GH-transgenic coho salmon have been shown to develop more rapidly embryologically and to emerge sooner from natal gravel redds, reducing their fitness by increasing their susceptibility to predation mortality (Devlin et al. 2004a; Sundström et al. 2005). GH-transgenic salmon also can develop faster through their freshwater phase, undergoing early smoltification (the physiological adaptation and migration from a fresh water to marine environment) in their first rather than second year of life (Devlin et al. 1994, 2000; Saunders et al. 1998). Thus, the timing of smolt migration is expected to influence the location where ecological consequences will occur (e.g., resource use in stream vs. marine environments), and would also affect the level of mortality experienced by transgenic individuals. Consistent with this, the present model predicts that transgene frequency in populations depends on the duration that transgenic fish remain in freshwater. Since survival improvement by early smoltification can offset viability reductions caused by the transgene phenotype at other stages, overall fitness of the transgenic phenotype can be enhanced. Evolution of such pleiotropic effects of the transgene and their interactions with the environment via selection of modifiers needs to be understood to facilitate accurate predictions of natural fitness and potential ecological consequences.

GH-transgenesis also can influence maturation age and adult body size in fish. For example, GH-transgenic coho salmon mature with a body size similar to wild type, but do so at 2 years rather than 4 years of age for wild-type fish reared in the laboratory (or 3 years for wild coho salmon) (Devlin et al. 1995, 2004a). In contrast, adult GH-transgenic rainbow trout mature at their normal age of 3 years, but at a much larger size (remarkably, 37- to 83-fold heavier) than their wild-type counterparts (Devlin et al. 2001). Larger adult fish (particularly male salmonids) are known to possess a breeding advantage in several salmonid species (Fleming 1998), prompting the suggestion that GH-transgenic fish may have a mating advantage facilitating the spread of the transgene. In medaka (*Oryzias latipes*), larger GH-transgenic males have been found to possess a mating advantage over wild-type males raised under the same conditions (Howard et al. 2004), providing support that GH-transgenic fish possess a reproductive fitness advantage. In GH-transgenic coho salmon, spawning ability was found to be inferior to wild type in laboratory trials in simulated streams (Bessey et al. 2004). However, because non-transgenic salmon reared in the same laboratory environment also showed impaired reproductive success, it was difficult to accurately partition genetic (transgene vs. wild type) and environmental (laboratory vs. nature) contributions to effects on breeding capabilities. Reproduction involves an extremely complex and flexible set of evolved morphologies, behaviours and physiologies, and indeed reproductive success in fish is not always associated with large body size. Several salmonid species possess an evolutionarily stable strategy where fast-growing males can reach maturation 'precociously' (at an age and size less than adult females) and employ a 'sneaker' or 'jack' strategy to fertilize eggs in spawning events involving regular males and females (Groot and Margolis 1991; Gross 1991). Such males transmit their genetic traits through generations faster than do regular males that mature at an older age. Thus, reductions in the age of maturation by GH-transgenesis has the potential to facilitate more rapid generational transmission of the transgene allele relative to other genetic variation in the population (Muir and Howard 1999, 2001). Indeed, Valosaari et al. (2008) have modeled how this evolutionarily stable mating strategy (i.e., precocious male maturation) in Atlantic salmon could modify effects GH-transgenic fish on populations and transgene persistence. Although GH-transgenic coho salmon do not show precocious maturation relative to transgenic females, both sexes do mature earlier than wild type. As expected, this compression of their life history has been predicted with the present model to facilitate transgene spread, even in the absence of other fitness advantages relative to wild type. Further, early maturation of transgenic fish at 2 years of age predicts elevated population levels under conditions (e.g. $V_T = 0.8$; $m_T = 2$) that otherwise cause a Trojan gene decline when transgenic fish mature at the normal 3 years of age.

Selection acting on transgenic individuals affecting the type and frequency of background genetic variation in populations has the potential to influence the phenotype of non-transgenic conspecifics as well. Loci that influence traits affected by the transgene are likely abundant in most natural populations, and variation at such loci would be under continual selection (directional and stabilizing) across the range

of fluctuating environments and selection regimes experienced by the organism. The entry of a transgene into a population could redirect selection upon this variation and consequent phenotypes away from that normally affording maximal fitness in wild-type individuals. The present model predicted that a modifier under selection by enhancing the fitness of transgenic fish can also affect non-transgenic populations that were otherwise maintaining variation at the modifier locus via balancing selection (e.g., via heterozygote advantage or from alternating directional selection in response to fluctuating environmental conditions). The presence of a transgene in a population could create a situation where selection of background genetic variation would be occurring counter to that favoured in wild-type individuals. This polarized selection could place a genetic load (Haldane 1957) on all individuals in the population by reducing the probability they would possess optimally selected background genetic variation for their genotype (transgenic or wild type). If counter selection of non-optimal background alleles does not occur rapidly in non-transgenic individuals, their suboptimal genotypes could further facilitate persistence of the transgene. In population fitness studies with Tribolium, a novel (eye-colour) allele was observed to cause higher individual fitness and therefore increased in frequency in one replicate population, but at the same time was associated with an overall decline in population size (Dawson 1969). This effect was attributed to a reproductive load arising in response to a "reshuffling of the gene pool" (e.g., selection of optimal background genetic variation) while adaption to the presence of the novel mutant allele occurred.

The simulation model presented here and those developed previously by other authors provide tools for understanding critical variables that play roles in influencing the persistence of transgenes in nature. However, we caution that using these theoretical constructs as tools to predict outcomes in nature is likely associated with a high level of uncertainty. For salmonids, the models developed to date, including the present one, do not fully reflect the complexities of their life histories, and do not incorporate the powerful environmental fluctuations that can be strong determinants of population size in salmonids. Further, ascribing parameter values that truly reflect those which would exist in nature cannot easily be validated based on the extant laboratory data alone. Complex pleiotropic effects of a GH-transgene are apparent across the full life history, and many of these phenotypic changes are strongly plastic and show non-parallel reaction norms across environments (Bessey et al. 2004; Devlin et al. 2004b; Sundström et al. 2005; Sundström et al. 2007). For risk assessments of fertile, highly-viable strains of transgenic salmon, comprehensive data sets from a broad range of conditions and life histories stages would be beneficial for prediction of potential consequences to ecosystems utilized by the species. The present modeling exercise has shown that understanding how transgene effects may evolve over time also will be critical for robust predictions of potential ecological consequences, and hence risk.

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