

Department of Biological and Environmental Sciences  
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# **Ecological Consequences of Genetic Modifications**

*– an Invasion Analysis Approach*

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Biosciences of the University of Helsinki, for public examination in lecture room 1041, Biocenter 2, on 7 March 2008, at 12 noon.

Helsinki 2008

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Cover design: Majka Borgström

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ISBN 978-952-92-3353-3 (pbk.)  
ISBN 978-952-10-4521-9 (PDF)

Yliopistopaino  
Helsinki 2008

# Ecological Consequences of Genetic Modifications – an Invasion Analysis Approach

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This thesis is based on the following publications:

- I** Aikio, S., Valosaari, K.-R., Ranta, E., Kaitala, V. & Lundberg, P. Invasion under a trade-off between density dependence and maximum growth rate. — Submitted manuscript
- II** Aikio, S., Valosaari, K.-R. & Kaitala, V. (2007) Mating preference in the invasion of growth enhanced fish. — *Oikos* (in press)  
doi: 10.1111/j.2007.0030-1299.16061.x,
- III** Valosaari, K.-R., Aikio, S. & Kaitala, V. Male mating strategy and the introgression of a growth hormone transgene. — *Evolutionary Applications* (conditionally accepted)
- IV** Valosaari, K.-R., Aikio, S. & Kaitala, V. (2008) Spatial simulation model to predict the Colorado potato beetle invasion under different management strategies. — *Annales Zoologici Fennici* 45: 1-14

The publications are referred to in the text by their roman numerals.

## Contributions

	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
Original idea	VK, ER	SA, KRV	KRV	SA, KRV
Study design	SA, KRV, VK,	SA, KRV, VK	KRV, VK, SA	KRV, SA
and analysis	PL			
Manuscript	SA, KRV, VK, ER	SA, KRV, VK	KRV, SA, VK	KRV, SA, VK

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# Contents

<b>Summary</b>	<b>6</b>
<b>1. Introduction</b>	<b>6</b>
1.1. Biological invasion	7
1.1.1. Invasiveness and invasibility	7
1.1.2. Consequences of invasions	8
1.2. Genetically modified organisms (GMO) – new alien species?	9
1.2.1. Increased invasiveness	11
1.2.2. Gene flow, introgression and genotypic displacement	12
1.2.3. Risk assessment of GMOs’	14
1.2.4. Current benefits and future expectations of GMOs’	14
<b>2. Aims of the thesis</b>	<b>16</b>
<b>3. Methods</b>	<b>16</b>
<b>4. Main results and discussion</b>	<b>22</b>
4.1. What makes a GMO an invasive alien? ( <b>I</b> )	22
4.2. Escape of growth hormone fish - a threat to natural populations through mating behaviour ( <b>II</b> , <b>III</b> )	24
4.3. Invasion of an exotic pest, Colorado potato beetle - GMO as a potential control strategy ( <b>IV</b> )	25
<b>5. Conclusions</b>	<b>26</b>
<b>6. Acknowledgements</b>	<b>28</b>
<b>7. References</b>	<b>29</b>

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# Summary

## 1. Introduction

The frequency of biological invasions is currently increasing due to globalisation and climate change (CBD 2008). Although invasions are frequent, only a small fraction of invading species usually become established, and only a small fraction of established invaders become detrimental to the invaded environment (Williamson & Fitter 1996a). However, this small fraction of successful invaders may lead to disruption and homogenization of natural communities, and in the worst case, to native species extinctions (Elton 1958, Carey *et al.* 1996, Wilcove *et al.* 1998, Huxel 1999, Zalba *et al.* 2000, Pimentel *et al.* 2001, Keane & Crawley 2002). Thus, biological invasions are considered as the greatest threats to biodiversity conservation, along with climatic change and habitat loss (CBD 2008). Invasive species have also caused enormous economic losses in agriculture and forestry as serious pest species (Pimentel *et al.* 2005).

Genetically modified organisms (GMOs) are modified to carry novel traits to, e.g., resist pests or cold or their growth is enhanced in comparison to their conventional counterparts. They may appear as new alien species if (1) they are able form a feral population in agricultural landscapes, (2) they are able to invade to natural habitats, or (3) they can hybridize with their relatives, either cultivated or wild, producing a new invasive hybrid form (Raybould & Gray 1994, Ellstrand & Schierenbeck 2000). Once GMOs are brought into contact with natural populations, it may lead to direct competition between GMOs and natural species, to hybridization and to introgression (gene flow between hybridizing populations when a hybrid backcrosses with one or both parent populations, Rhymer & Simberloff 1996) of transformed traits into nature. In the worst case, invasion may result in competitive exclusion of native species, the population declines through outbreeding depression, or displacement of the native genotypes by the introduced ones through genetic assimilation.

Scientific ecological risk assessment should precede any GMO introduction for agriculture, aquaculture, or forestry practices, or whenever GMOs are brought into contact with natural ecosystems (EU 2002). To assess the risks of GMO invasion into natural populations, it is important to be able to predict both the invasion probability of the GMOs' (exposure) and the impact a successful invasion may have in the invaded ecosystem (magnitude of hazard). Ecological invasion analysis may be a useful tool for both purposes addressing the following questions: What are life history traits likely to make a species invasive or further increase its invasiveness? Why some habitats are more invulnerable (vulnerable to invasions) than others? What is the outcome of successful invasion?

## 1.1. Biological invasion

### 1.1.1. Invasiveness and invasibility

There are three main stages of the invasion process, (1) initial colonisation of a new habitat, (2) establishment of a self-sustainable population, and (3) population increase and spread. Each requires different life history traits of a species to make it invasive (Mollison 1986, Kolar & Lodge 2001, Rejmánek *et al.* 2003). During the first stage, the most important trait is dispersal ability, to enable the colonisation of a new habitat, but also competitiveness to succeed initially (Mollison 1986). Immigration of an invading species may occur naturally, where a species extends its' distribution, e.g., due to climate warming, but often it is vectored by humans. Human vectored dispersal of invasive species may be either purposeful, such as introductions of a new crop species, or it may be accidental, such as species dispersing over seas within cargo ships' ballast water (Mack *et al.* 2000, Carlton & Ruiz 2005). Secondly, an invader has to be competitive to be able to persist, and thirdly, efficient in reproduction (early reproduction, high offspring production) and highly dispersive, to be able to spread (Mollison 1986, Kot *et al.* 1996, Neubert & Caswell 2000, Kolar & Lodge 2001, Rejmánek *et al.* 2003). Competitiveness is involved in many stages of the invasion process and it is an important determinant of invasion success. Competitive superiority can be gained by, e.g., more efficient utility of common limiting resources, more efficient reproductive capacity, or better avoidance of a shared predator or pathogen.

Baker (1974) presented a list of weedy characteristics of plants that can potentially increase the invasion ability of a species. In theory, fitness evaluations should predict the invasion success. For instance, if an invading plant produces fewer seeds (lower fecundity) or has lower survival ability than the resident, it will be less likely to establish in natural communities. However, in a study of Bergelson (1994), the genotype with inferior seed production in *Arabidopsis thaliana* invaded natural habitats as successfully as did the wild genotype. This could occur whenever population size is limited by factors other than reproduction ability. Thus, an invasion analysis based on life history traits alone may sometimes fail in predicting the invasiveness of a species in a new environment (Williamson & Fitter 1996b).

Invasion success is not only dependent on the species' invasiveness but community invasibility is also an important determinant. The evolutionary history of natural disturbance, along with human impact, has an important role in the susceptibility of a region to invasion by exotic species (Higgins & Richardson 1996). Disturbance usually decreases competition, releases resources and therefore increases the probability of invasion (Grime 1973, Crawley 1986, Vilà & Weiner 2004). Community invasibility has also been found to depend on the number of species it contains, species rich sites being more resistant to invasions (Elton 1958). However, contradictory results have been presented and the invasibility of the habitats is probably more due to effects on resource availability than species richness itself (Stohlgren *et al.* 1999, Stachowicz *et al.* 2002). In addition, invasion can also result without superior competitive ability of the invasive

species. The enemy release hypothesis predicts that exotic species, when introduced to new habitats, may not suffer from the regulation by natural enemies' as much as native species, and therefore exotics could increase in distribution and abundance (Keane & Crawley 2002).

Invasion (gene flow) may also occur at the gene level through hybridization between sexually compatible native and introduced populations (Rhymer & Simberloff 1996, Ellstrand & Schierenbeck 2000, Petit 2004, Lambrinos 2004). For example, spontaneous hybridisation with wild relatives appears to be a general feature of many of the world's important crops (Klinger & Ellstrand 1994, Ellstrand *et al.* 1999). The term 'cryptic invasion' has been used to describe the situations when non-native genotypes spread (movement of alleles) within the same species (Saltonstall 2002, Petit 2004). The fitness of hybrids and the spread and persistence of new genes into the invaded population is dependent on the extent of selective advantages it brings along. Neutral or advantageous genes will tend to persist (introgress) but neutral genes should not influence the population dynamics of the target, whereas harmful genes that reduce the fitness of target individuals should be removed due to natural selection (Klinger & Ellstrand 1994). The exception is the situation when the invasion to a locally rare population is continuous, leading to repeated hybridization and introgression of genes that may have negative effects on fitness (Conner *et al.* 2003). Also, a gene thought to be neutral may be under selection in different environments (Hails 2000).

### 1.1.2. Consequences of invasions

Invasion of an alien species leads to an interaction with resident species which may either prevent or facilitate the establishment. The interaction may take one of several forms, such as competition, predation, parasitism, mutualism, or hybridization.

Competition between the resident and the invader may lead to three alternative direct outcomes: (1) the invader goes extinct, (2) the resident species or population goes extinct, (3) the invader and the resident coexist, or (4) both go extinct. Interspecific competition is an important process of plant invasion and competitive exclusion also seems to be the most important factor resisting the establishment of alien species (Keane & Crawley 2002). Displacement of the native species by a competitively superior invader, leading to (local) extinction of the native, is one of the most severe direct risks of biological invasions (Wilcove *et al.* 1998, Williamson 1999). A famous example is the fire ant (*Solenopsis invicta*) invasion in south eastern United States where it has competitively excluded native ant (70 % reduction in native ant species richness) and other arthropod species (Porter & Savignano 1990). However, the role of competitive exclusion in native species extinctions after invasion has been recently challenged by Davis (2003) and Gurevitch and Padilla (2004) and they suggest that extinctions of native species are rarely solely due to the competitive exclusion by invading species, but there are almost always other factors, such as predation, pathogens or hunting, involved (reviewed in Sax *et al.* 2007).



Hybridization between invading species or genotypes and a resident relative may also lead to extinction of either genotype, to coexistence of parent genotypes, but also to the persistence of the new hybrid form (Huxel 1999). The direct risks of gene flow between invasive and native species or populations are genetic assimilation and demographic swamping where invading genes displace the native ones (Huxel 1999, Wolf *et al.* 2001). Genotypic assimilation leading to genotypic displacement may result from interbreeding between native and invading genotypes when the latter is favoured by selection. Demographic swamping results from the continuous immigration of a non-native genotype leading to fixation of invading alleles (introgression) into the native population and finally displacing the native alleles (Huxel 1999). As a result a great amount of genetic variation is lost, homogenizing the species or populations (Huxel 1999, reviewed in Ellstrand *et al.* 1999).

It has been claimed that hybridization may facilitate the evolution of invasiveness by producing totally novel genotypes, increasing genetic variability, producing heterotic genotypes, or by reducing the frequency of detrimental alleles by recombination (Ellstrand & Schierenbeck 2000). Hybridisation between invasive and native species or populations can thus be a source of variation and it may also lead to speciation (Mooney & Cleland 2001, Hails & Morley 2005). However, interbreeding between native and invading individuals may also lead to outbreeding depression if the fitness of hybrids is reduced. In the worst case, this may lead to total population extinction (Levin *et al.* 1996, Rhymer & Simberloff 1996, Hutchings 1991, Ellstrand *et al.* 1999, Muir & Howard 2001). For example, it has been shown that frequently escaped farmed fish from aquaculture hatcheries (McGinnity *et al.* 2003, Fiske *et al.* 2006) may threaten natural populations of their wild conspecifics or relatives by competing for the same resources, especially for food, or by hybridizing with them (Hutchings 1991, Einum & Fleming 1997, McGinnity *et al.* 1997, 2003, Madeira *et al.* 2005, Hindar *et al.* 2006).

Invasions may also lead to severe community level changes, e.g., disturbing nutrient cycling or fostering more frequent fires (Mack 2000). The changes may further lead to cascading effects when one change in the community leads to another and in the worst case to the disruption of the whole ecosystem (Crooks 2002, Cox 2004, Lundberg *et al.* 2000, Enberg *et al.* 2006).

## **1.2. Genetically modified organisms (GMO) – new alien species?**

“Organisms with novel combinations of traits are more likely to play novel ecological roles, on average, than are organisms produced by recombining genetic information existing within a single evolutionary lineage.”

*Tiedje et al. 1989*

Genetic modification (GM) is a branch of biotechnology where new genes are transferred, e.g., by *Agrobacterium* mediation, microinjection, electroporation or particle gun bombardment methods, into a target organism's genome (reviewed in Babu *et al.* 2003). With this technique an organism's own gene expression can be increased or decreased

specifically. Another option is that the introduced genes can be totally novel, unfamiliar genes to the target species originating from other taxonomic groups (Snow & Palma 1997). For example, the second most used genetic modification today is to transfer the toxicity gene of *Bacillus thuringiensis*, the common soil bacterium and natural pathogen of many insects, to crop plants (i.e. maize, cotton) to make them resistant against insect pests (Tabashnik *et al.* 2003, James 2006). Such crops are referred as Bt crops.

Genetic modification is not fully comparable with selective breeding. Conventional selective breeding results in the cumulative effect of many genes, whereas in genetic modification the result is only the major effect of one gene and it may also involve pleiotropic effects (secondary phenotypic effects of a single genetic alteration) (Tiedje *et al.* 1989, Wolfenbarger & Phifer 2000, Muir & Howard 2002). This is the main difference between GMOs and conventionally bred species.

Possible ecological effects of GMOs' are numerous and they can be either negative or positive (see Box 1). Genetic modification changes the phenotypic properties of the modified individual and it may become totally novel with respect to natural conspecifics. This may lead to changes in organisms' relations with other species which they interact with (Wolfenbarger & Phifer 2000). Adverse effects of genetically modified species may occur if they are able to persist or invade into natural communities, becoming new exotic species. Alternatively, they can hybridize with other cultivars or natural relatives after escaping from cultivation and the hybrid becomes invasive or weedy (Raybould & Gray 1994, Ellstrand & Schierenbeck 2000). In addition genetically modified organisms may potentially cause harmful non-target effects on different trophic levels, like on herbivores, pollinators, target species' natural enemies or on soil organisms (Firbank *et al.* 1999, Losey *et al.* 1999, Brooks *et al.* 2003, Firbank 2003, Romeis *et al.* 2004, 2006, Marvier *et al.* 2007). The wide scale use of the Bt crops has also raised concern about the resistance development of the target species against Bt toxin, resulting in even greater difficulty in controlling pest populations (Cerdeira & Wright 2002, Andow & Zwahlen 2006). For example, the Bt potato modified to control a severe pest of the potato, the Colorado potato beetle, has been withdrawn from the market and cultivation partly for that reason (Boiteau 2005).

### **Box 1. Potential benefits and risks of GMOs'**

#### **Benefits:**

- Novel biotechnical products
- Increased per hectare yields
- Decreased pesticide use (**IV**)
  - reduced environmental impacts
  - reduced negative health effects
- Reduced wide-spectrum herbicide use
- Reduced tillage need
  - reduced erosion and water loss
  - increased soil organic matter
  - reduced emission of greenhouse gasses

Modified from Wolfenbarger and Phifer (2000), Brookes and Barfoot (2006)

#### **Risks:**

- Direct or indirect toxic or allergenic effects on human or domestic animals
- Direct or indirect toxic effects on non-target species
  - loss of biodiversity
  - altered community functions
- Increased invasiveness (**I**)
  - weeds of agriculture
  - invasive in natural habitats
- Gene flow by interbreeding between related taxa (**II, III**)
  - hybrid vigour (hybrid offspring more weedy or invasive)
  - outbreeding depression
- Horizontal gene flow
- Evolution of resistance (**IV**)
- Changes in farm practices leading to changes in biodiversity

Modified from Wolfenbarger and Phifer (2000), Snow *et al.* (2005)

#### **1.2.1. Increased invasiveness**

Changes in the adaptive characteristics of transgenic organisms can make them better competitors, predators or less vulnerable as prey if the trait introduced is totally novel or gives fitness a advantage over wild conspecifics (Muir & Howard 2002). These changes can make a species invasive and probably a threat to agricultural or natural ecosystems by becoming an agricultural weed or invasive to natural habitats (Crawley *et al.* 1993). As an indirect effect, genetic modifications could create new invaders by increasing the resistance of herbicides in crop plants or develop tolerance to pest resistant crops in agricultural pests, enhancing the effects of any existing weeds or pests (Tiedje *et al.* 1989, Pretty 2001, Cerda & Wright 2002, Andow & Zwahlen 2006).

The competitiveness of transgenic organisms may be altered in various ways. As an example, the escape of growth enhanced, disease resistant or cold tolerant transgenic fish,

which have increased fitness compared to native wild species, could be risky because of their competitive advantage over wild species (Devlin *et al.* 1999, Maclean & Laight 2000, Martin-Smith *et al.* 2004). Effective invasion ability is also a key trait leading to the development of potential new weeds of transgenic crops (Conner *et al.* 2003). If the target species of genetic modification already has many weedy characteristics, it would be hazardous to introduce some fitness enhancing gene in its genome (Parker & Kareiva 1996). This could result in the evolution of a “superweed”, which would be problematic to control.

Introduced transgenes may be differently expressed under different environmental conditions. Hence, predictions about the invasiveness or competitiveness of transformed individuals in the wild cannot be based on their performance in, e.g., laboratory conditions only (Devlin *et al.* 2006, Sundström *et al.* 2007). If the trait introduced is, for example, herbicide resistance, it is probably beneficial only in situations where herbicides are used. Similarly, pest tolerance is probably beneficial only when the pest is present. However, increased allocation to one trait often brings costs due to limited resources. For example, growth enhancement in salmonids produced by transgene technology has been shown to reduce their predator avoidance ability and offspring viability (Devlin *et al.* 2004, Sundström *et al.* 2004). It is not straightforward to ascertain whether the seemingly altered fitness of the transgenic organism will result in better invasion ability (Hails 2000). As Crawley (1997) pointed out, competitive ability is not a genotypic trait, but rather a phenotypic trait, and it also depends on the environment and on other competitors.

### 1.2.2. Gene flow, introgression and genotypic displacement

The most problematic GMOs are those whose ability to hybridise with wild relatives is highly probable because of overlapping geographical distribution and high reproductive compatibility (Raybould and Gray 1993). In addition to invasiveness, gene flow from GMOs to their wild relatives is one of the greatest concerns in the use of transgenic organisms. Gene flow from GMOs to natural species may occur through hybridisation in higher organisms (vertical gene flow) or through conjugation (by plasmids), transduction (by viruses) or transformation (free DNA) in micro organisms (horizontal gene flow) (Tiedje *et al.* 1989, Crawley *et al.* 1993, Hails 2000, Pretty 2001, Tepfer 2002). The result of gene flow could be the introgression of the engineered genes into the genome of wild relatives (Ellstrand *et al.* 1999, Desplanque *et al.* 2002). Hybrid offspring may become weedy or more invasive (Crawley *et al.* 1993, Hails 2000) or they may produce new pathogens by recombination (Pretty 2001). Concern should be focused on the fitness enhancing effects of the transgenes, but also on the extent of the gene flow and introgression (Raybould and Gray 1993).

The major indirect risk of gene flow from GM crops to wild populations is the evolution of weedy characteristics by the transfer of selectively advantageous crop alleles (or novel GM alleles) to weedy (or wild) relatives (Klinger & Ellstrand 1994, Levin *et al.* 1996). Hybridisation with wild relatives has been implicated in the evolution of more aggressive weeds for seven of the world’s 13 most important crops (Ellstrand *et al.* 1999).

To evolve as a weed, the hybrids must also be able to persist and reproduce (Klinger & Ellstrand 1994). Novel traits, like herbicide tolerance may have positive impact on fitness, which could lead to weediness if introgressed into wild population, but these traits may also bring costly pleiotropic effects (Bergelson & Purrigton 1996). Despite the pleiotropic effects, the hybrid may turn out to be weedy. After the formation of the hybrid, natural selection will tend to increase its fitness rather than decrease it, by reducing the costs associated with the novel genes (Tiedje *et al.* 1989).

Gene flow between transgenic individuals could also bring new, unexpected problems for farming practices. Crop to crop hybridization may lead to more severe weeds when, for example, many herbicide tolerance genes aggregate to the hybrid and there are no costs of multiple tolerances (Hall *et al.* 2000, Beckie *et al.* 2003, Simard *et al.* 2005). In Canada double herbicide tolerant types of canola (*Brassica napus*) individuals have already developed due to pollen flow between different herbicide tolerant canola varieties (Beckie *et al.* 2003). Hybridization is also problematic when growing both organic and GM varieties, or when turning to organic or conventional farming after GM farming (Perry 2002).

An example of the weed evolution by hybridization between transgenic and wild relative is provided by Snow *et al.* (2003) who studied the weediness potential of wild and herbivory tolerant (especially lepidopteran herbivory) transgenic (Bt) sunflower hybrids. Wild sunflower is a weed in crop sunflower fields and it is known to easily hybridise with the crop variety. The introduction of a Bt transgene may have dramatic effects on herbivory and fecundity in wild sunflowers. This fecundity benefit may suggest that the Bt transgene has a selective advantage over the wild type and for this reason it could quickly spread in wild sunflower populations. This was the first study where the formation of a more weedy type (superweed) was shown to be possible, and indeed, probable.

Theoretical models have shown that the hybridization of growth enhanced transgenic fish with its natural conspecifics may lead to total population extinction through outbreeding depression but these models lacked a density dependent regulation factor (Muir & Howard 1999, 2001). Muir & Howard (1999, 2001) found in their modelling study with Japanese medaka fish that escape of the growth enhanced GM fish to the natural population may cause an extinction risk because of the transgene's pleiotropic effects. They studied how six different fitness components affected the predicted changes in transgene frequency after transgene release to the natural population. The transgene could spread in populations, even if it also brought costs, like lowered viability of transgenic offspring, but it also had positive effects on the other fitness components, like male mating success. This may cause two hazards: extinction resulting in the local elimination of the conspecific population or invasion involving ecosystem disruption, when GM individuals replace their wild type counterparts (Muir & Howard 2002). Extinction could happen because of opposing pleiotropic effects of the transgene (on net fitness components). Invasion could be a result if the transgene brings some novel or enhanced fitness advantage with it over the wild type conspecifics (Muir & Howard 2002).

### 1.2.3. Risk assessment of GMOs'

Risk is a function of hazard and exposure, where the hazard is the severity of the potential harm and the exposure is the likelihood that the hazard is realized (Damgaard & Lokke 2001, Muir & Howard 2002, Conner *et al.* 2003, Raybould & Wilkinson 2005). Nap *et al.* (2003) suggested that risk assessment of GMOs' should thus consider the possibility, probability and consequences of harm on a case-by-case basis and the impact of non-use should also be added to the evaluation. It is also recommended that the risks involved with GM plants are compared to the risks of conventionally bred plants (Barton & Dracup 2000, Nap *et al.* 2003, Conner *et al.* 2003).

Sutherland and Poppy (2005) suggested that the starting point of the risk assessment of GMOs' should be the assessment of the possible endpoint of their use. Tiered risk assessment, where the first step is the worst case study usually conducted in the laboratory (e.g., Losey *et al.* 1999, monarch study), the second step is small scale field study, and the third step is large scale field study (e.g., FSE, Firbank *et al.* 1999, Firbank 2003), is recommended to assess the probability of the exposure and harm caused by GMO use (Poppy 2000, Sutherland & Poppy 2005). If exposure or harm is not realized in some step of the tiered assessment the testing may be stopped there and the GM product can be assessed as safe.

However, long-term studies are needed to assess the environmental impacts of the GMOs released to nature, including both spatial and temporal replications. Also the importance of post-approval monitoring over several years is emphasised by Schuler *et al.* (1999), Barton & Dracup (2000) and, Kauppinen *et al.* (2003), especially when GMOs are grown on a large commercial scale, to assess the possible long term or cumulative effects of the GMOs. However, these demands are difficult to meet and the duration of the experiments are often much shorter than the life spans of the test organisms.

### 1.2.4. Current benefits and future expectations of GMOs'

The first genetically modified crop plants for commercial use were planted in the US in 1996 and in ten years the worldwide area of GM crops has increased to 102 million hectares (James 2006). The six biggest GM farmlands were USA, Argentina, Canada, Brazil, China and Paraguay in 2006. The main GM crops cultivated are soy, maize, cotton and rapeseed (canola) and 29 % of these crops were GM varieties in 2005 (Brookes & Barfoot 2006). The dominating transferred traits are herbicide tolerance and pest resistance (mainly Bt).

The main aim of farming genetically modified organisms is to increase the efficiency of food production and to reduce the environmental impacts of food production practices (or industrial processes) on the environment (Box 1). The most significant benefit of the use of herbicide tolerant crops is the possibility to reduce the use of herbicides (Freckleton *et al.* 2003, Squire *et al.* 2003) enabling us to move from conventional tillage system to low tillage system (Brookes & Barfoot 2006). The volume of herbicides used with GM crops

has decreased 4.1 % in comparison to conventional farming from 1996 until 2006 (Brookes & Barfoot 2006).

Genetically modified pest or disease resistant crop plants are beneficial in farming practices, because the use of the synthetic, wide spectrum pesticides and insecticides can be reduced (Huang *et al.* 2005, James 2006). For example, within the cultivation of Bt cotton the reduction in the insecticides used has been 19 % from 1996 until 2006 (Brookes & Barfoot 2006). The reduced insecticide use also reduces negative environmental and health effects of pest control (Ferré & van Rie 2002, Shelton *et al.* 2002, Ammann 2005, Huang *et al.* 2005). An additional benefit of the herbicide tolerant and pest resistant crop plants is the reduction of green house gas emissions due to reduced insecticide and herbicide applications and reduced tillage.

In the EU, the only crop commercialized is Bt maize (GMO Compass 2008) and the public acceptance of GM food and cultivation is still low. (Conner *et al.* 2003, Nap *et al.* 2003, GMO Compass 2008). Opposition against GM crops in Europe increased in the late 20<sup>th</sup> century when research from the US showed that transgenic Bt-maize might cause the death of non-target monarch butterflies (Losey *et al.* 1999, Jesse & Obrycki 2000, Oberhauser *et al.* 2001). These results, and the lack of knowledge of the environmental and health impacts of GMO's, led to *de facto* moratorium on commercial licensing of new GMO products (which commenced in June 1999) in the EU (Shelton & Sears 2001). The moratorium was dissolved in May 2004 and nowadays six countries, Spain, France, Czech Republic, Portugal, Germany, and Slovakia, have commercial GM crop cultivation. However, many other countries in Europe, including Finland, have deliberately released GMOs for field trial purposes (GMO Compass 2008).

Although herbicide tolerance and pest resistant crop plants are the dominant commercial applications of GMOs today, e.g., virus, bacteria, and fungi resistant, drought tolerant and nutritionally enhanced crop plants, and also GM-trees with lowered lignin production to enable reduced chemical use in the paper industry, are under ongoing research (Pilate *et al.* 2002, Herdt 2006).

The research on GM applications does not only include plants, but farmed animals. For example, research on the production of fish that grow faster than their conventionally bred siblings has been going on for two decades (Hulata 2001) and GM fish are already waiting for licensing to be commercialized in US (Atlantic salmon), China (common carp), and Cuba (tilapia) (Hallerman *et al.* 2007). In addition to commercial applications, for example, goldfish, loach, medaka and zebrafish have been engineered for modelling studies (Hulata 2001), together with over 35 other fish species (Zbikowska 2003). While transgenic food fish are still waiting for licensing to become commercial, the first commercial transgenic pet (aquarium fish) in the USA is a red fluorescent protein (RFP) transgenic zebra danio (*Danio rerio*) (Cortemeglia & Beitinger 2006).

The increased fitness of transgenic organisms in comparison to conventional or wild relatives is usually considered as a potential threat, as discussed earlier. However, increased fitness may also be an intended trait in applications where transgenic individuals are supposed to displace the native ones, such as with transgenic malaria resistant mosquitoes (Marrelli *et al.* 2007).

## 2. Aims of the thesis

In this thesis I will concentrate on different aspects of GMO invasions to wild: how gene modification may increase species invasiveness (**I, II, III**) and, on the other hand, how gene modification could be utilized to manage exotic species invasions (**IV**). I aimed to answer to following questions:

1. What makes a GMO a superior competitor and enables its invasion to the population of a natural species or conventional cultivar? What is the competitive outcome of invasion (**I**)?
2. Could escaped or released fish with growth hormone transgene invade and spread in the wild population via hybridisation (**II,III**), would the different mating strategies of genotypes affect the introgression probability of the transgene into a wild population (**III**), and what would the outcome be of successful invasion (**II,III**)?
3. What life history traits are the most important determinants of the invasion probability of an exotic insect pest and could cultivation of a genetically modified insect resistant host plant give an additional benefit in the management of this invasive pest (**IV**)?

## 3. Methods

The main method used throughout this thesis is theoretical modelling which combines life-history evolution, population dynamics, and population genetics to apply and develop invasion theory. The models are used to analyze the theory of invasion dynamics in general (**I, IV**), the effects of genetic modification on various life history traits and the competition between transgenic organisms and natural populations (**I, II, III**).

I have used different population models to describe the dynamics of the study populations within this thesis. The Maynard Smith and Slatkin (MSS) population model was used to study the role of density dependent competition in the invasion of an exotic species (or a GMO) in Chapter **I** (Maynard-Smith & Slatkin 1973). The MSS model for the change in population density ( $N$ ) between subsequent year's  $t$  and  $t+1$  is

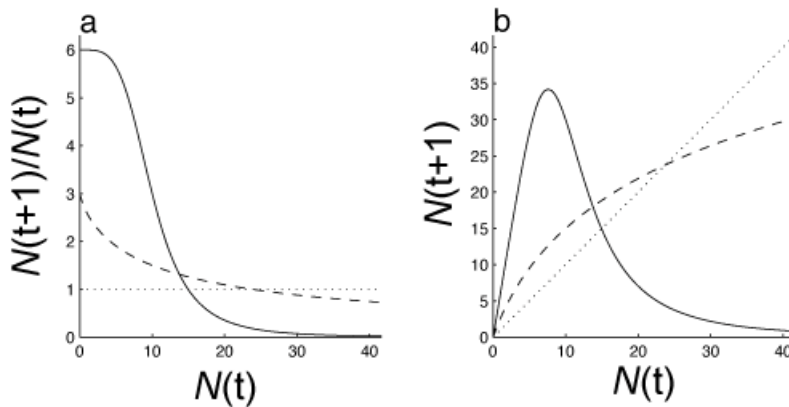
$$(1) \quad N(t+1) = \frac{\lambda N(t)}{1 + [aN(t)]^b}$$

Parameter  $\lambda$  is the maximum annual growth rate of the population (the intersection of the curve at the y-axis, Fig 1a). An increase in  $\lambda$  increases the equilibrium density of the population, but may also lead to unstable dynamics. The strength of density dependence ( $a$ ) scales equilibrium density, but it has no effect on stability of the population dynamics.



Parameter  $b$  determines the shape of the density dependence. The effect of parameter  $b$  on equilibrium population density depends on the value of parameter  $a$ , i.e., a population with a high value of  $b$  can have either higher or lower equilibrium density depending on the strength of density dependence.

I chose the MSS model because it allows the population growth rate and the weight of density dependence to be varied independently, whereas the more frequently used and simpler Logistic or Ricker models have both growth and density dependence determined by the same parameter. Within the MSS model the density dependence may be varied from undercompensatory (contest competition, stable population dynamics) to overcompensatory (scramble competition, cyclic or chaotic population dynamics) with different values of parameter  $b$  (Nicholson 1954) (Fig. 1). High values of  $b$  lead to overcompensatory density dependence and consequently unstable population dynamics, including chaos.



**Figure 1.** (a) Density dependence of per capita growth rate and (b) recruitment curves of the population for the fast growing species with scramble type density dependence (solid line) and for slow growing species with contest type (dashed line) density dependence.

The MSS model was extended as a two species system. I analysed whether one of the species, called the intruder (an alien species or GMO) is able to invade the system where the other species, called the resident, is initially more abundant. I chose the parameter values for the resident population to represent three qualitatively different dynamics: point equilibrium (stable), cycle and chaos. The intruder population's parameters were expressed in relation to the resident and the intruder population's dynamics was analyzed for each of the qualitatively different cases of resident dynamics over a wide parameter space. The effect of the resident population's density on the intruder population's growth rate  $G[N_R(t),0]$  can be read from Figure 1a by replacing population density  $N(t)$  on the x-axis by the *resident* population density  $N_R(t)$  and replacing recruitment of the population  $N(t+1)/N(t)$  by the recruitment of the *intruder* population  $N_I(t+1)/N_I(t)$ .

Traditional invasion analysis, where the resident population is at the equilibrium and the initial density of the intruder is negligible, was conducted by analytically solving the model for cases where the intruder may increase when rare. However, I was specifically interested in the effect of intruder population's initial density on the success of invasion. This was the main motivation of choosing a simulation approach in the case of the two populations having the same equilibrium density instead of the traditional invasion analysis, where the intruder population's density is ignored.

Hybridization and the introgression of the genetically modified traits to nature were studied in Chapters **II** and **III** with a model that combines population dynamics with the inheritance of the transgene. Introgression of the transgene was traced by two alleles (wild:  $A$ , GMO:  $a$ ) in one locus and the offspring frequencies followed Mendelian inheritance. The transition matrix  $\mathbf{A}$  between genotypes ( $W$  = wild,  $H$  = hybrid,  $G$  = GMO) is given as:

$$(2) \quad \mathbf{A} = \begin{bmatrix} f_W + \frac{1}{2}f_H & \frac{1}{2}f_W + \frac{1}{4}f_H & 0 \\ \frac{1}{2}f_H + f_G & \frac{1}{2}f_W + \frac{1}{2}f_H + \frac{1}{2}f_G & f_W + \frac{1}{2}f_H \\ 0 & \frac{1}{4}f_H + \frac{1}{2}f_G & f_G + \frac{1}{2}f_H \end{bmatrix}$$

where  $f_W$ ,  $f_H$  and  $f_G$  are the frequencies of matings gained by each genotype in the population. The population dynamics followed the deterministic discrete time model where the population density vector  $\mathbf{n}$  changes during subsequent years  $t$  and  $t+1$  as

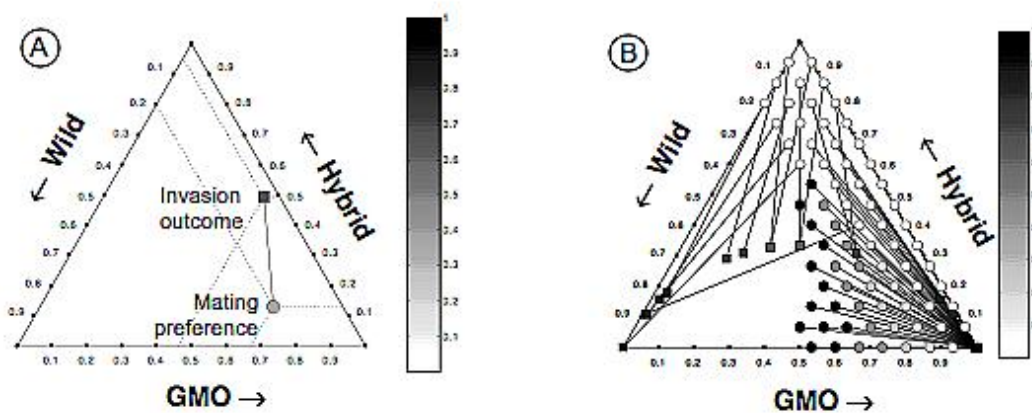
$$(3) \quad \mathbf{n}(t+1) = \frac{\mathbf{A}\mathbf{n}(t)}{1 + \mathbf{b}^T \mathbf{n}(t)}$$

where the vector  $\mathbf{b}$  expresses the genotype-specific contribution to density dependence and the superscript  $T$  denotes the transpose of the vector. The effect of density dependence is similar to the Beverton-Holt population model, which is well-established in the modelling of fish populations (Wootton 1998), and does not produce inherently unstable population dynamics (Beverton & Holt 1957). Other life history traits; survival, fecundity, preference in mating, mature parr proportion and mature parr mating success, were implemented to the model according to each case studied in Chapters **II** and **III**. The transition matrix  $\mathbf{A}$ , here presented simply for three genotypes, was modified in Chapter **III** to represent both sexes and two life history strategies for males (early maturing parr and anadromous) within the genotypes. Anadromous males experience one year delay in reproduction but for females there is only one life history strategy (i.e., age class) modelled and thus no delay in reproduction.

I initiated the invasion analysis for both Chapters **II** and **III** by simulating the wild genotype and allowing it to reach its equilibrium density. This was followed by the

introduction of a transgenic genotype with the initial densities ranging from the extinction level ( $10^{-6}$ ) to the equilibrium density of the wild genotype. To see the long term outcome of the attempted invasion I simulated the systems for 1000 or 1500 time steps.

In Chapter II, the different mating preferences of wild, hybrid and GMO genotypes form a triangular parameter space (see Fig. 2 for the interpretation of the results). I investigated the requirement and outcome of invasion for the entire parameter space of mating preference for cases where (1) genotypes are identical (2) one of the vital rates (fecundity and survival) differs between genotypes, and (3) vital rates are traded-off with each other (e.g., trade-offs reviewed by Stearns 1989). I also applied the model in a reanalysis of the effect of growth enhancement in Japanese Medaka (*Oryzias latipes*, Box 2) from the data of Muir and Howard (2001).



**Figure 2.** (A) Interpretation of results presented in triangle diagrams. The three sides of the triangle display genotypes proportions, which sum to the value of one. The dotted lines indicate how each marker is projected to the axes of the triangle. The location of the circle inside the triangle indicates the mating preference of each genotype. The scale of grey at the circular marker indicates the required initial density in relation to the pre-invasion density of the wild type. The location of the square indicates the frequency of the genotypes at the end of simulation, 1000 time steps after the invasion. The scale of grey in the square marker indicates population density in relation to the pre-invasion density of the wild genotype. (B) Invasion under different mating preference of genotypes when the survival of the hybrid genotype is higher than wild and transgenic genotypes but the other vital rates are identical within genotypes.

The model species in Chapter III, Atlantic salmon (*Salmo salar*, see Box 2), is known to have two male mating types, anadromous males and mature parr (sneakers). To understand the role of mature parr mating strategy in invasion I firstly analysed the effect of the proportion of mature parr and mating success on the invasion probability of the transgene into the wild population. Secondly I investigated how changes in the genotypes' mating preferences, mature parr proportions, and mature parr mating success affect the introgression. The hybrid phenotype may have an important role in the introgression of a transgene. Thirdly I analyzed five possible cases where hybridization of wild and transgenic genotypes leads different outcomes in hybrids: (1) all genotypes are identical, (2) the transgene is additive, (i.e., the heterozygote is an intermediate of the homozygotes),

(3) the heterozygote has an advantage over the two homozygotes, aka. heterosis, (4) the transgene is recessive, (i.e., heterozygote is like the wild genotype), or (5) the transgene is dominant (heterozygote is like GMO genotype).

### Box 2. Model organisms

#### Japanese medaka (*Oryzias latipes*, Temminck & Schlegel, 1846) (II)

- **Environment:** fresh and brackish water
- **Distribution:** Japan, Korea, Taiwan and China
- **Experimental environment:** cultured in lab
- **Transgenic line:** human growth hormone (hGH) gene with salmon promoter (psGH)
- **Life cycle:** longevity in nature: 1 year, in lab: 3-4 years; females breed daily through breeding season
- **Importance:** experimental research organism

Source: Muir & Howard 1999, 2001

#### Atlantic salmon (*Salmo salar*, Linnaeus, 1758) (III)

- **Environment:** freshwater; brackish; marine
- **Distribution:** temperate and arctic zones in northern Atlantic Ocean
- **Life cycle:** juveniles in freshwater streams, anadromous migrate to sea to grow and mature or male juveniles may premature in natal stream (mature parr), anadromous return after 1–4 years to breed in natal streams and then return back to sea. Small proportion may re-reproduce.
- **Importance:**
  - commercial in fisheries and aquaculture, game fish
  - red list status: Lower Risk/least concern (LR/lc)

Source: FishBase ([www.fishbase.org](http://www.fishbase.org))

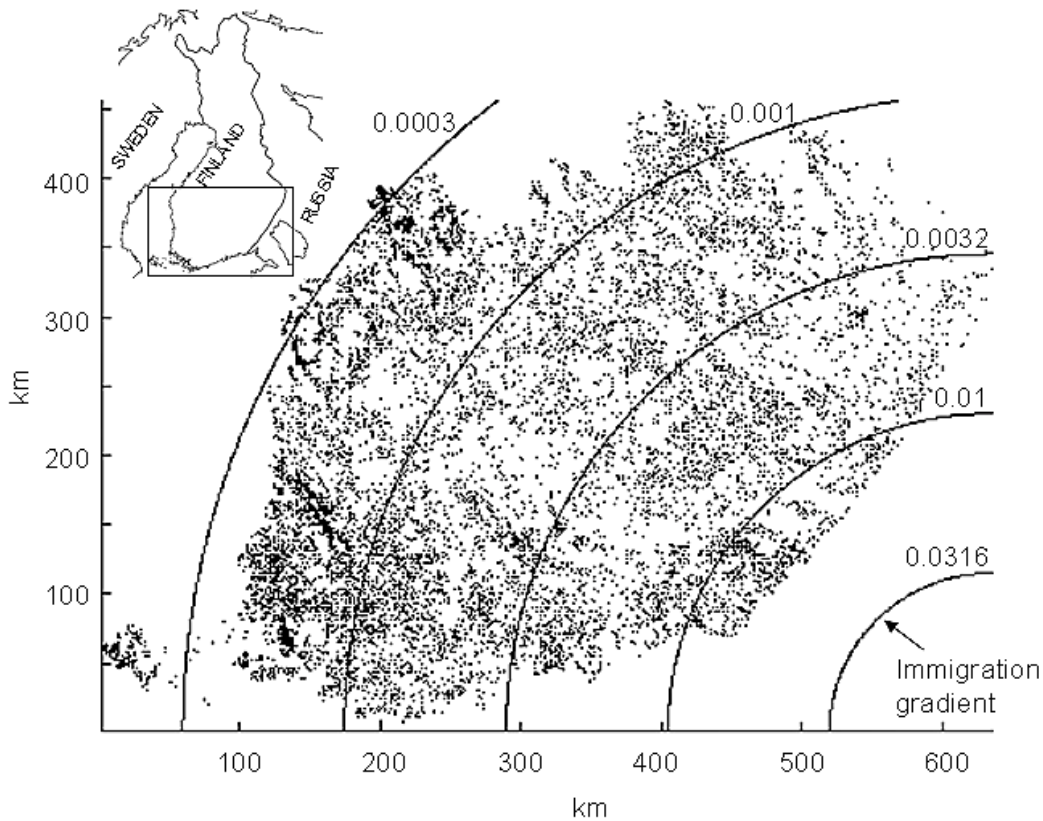
#### Colorado potato beetle (*Leptinotarsa decemlineata*, Say) (IV)

- **Distribution:** originally from Mexico, spread through North America, Asia and Europe, except Scandinavia, Great Britain and Channel Islands.
- **Life cycle:** 1 to 4 generations per year, four larval stages, pupate in soil, adult diapauses over unfavorable (winter) season and female lays eggs in batches (10-30 in each, overall ca. 2000 per female) on Solanaceae host plants. Both larvae and adults feed on host plants.
- **Importance:** most severe worldwide pest species of many solanaceous crop plants, especially potato

Source: Hare 1990

In Chapter IV I investigated the invasion potential of the Colorado potato beetle (*Leptinotarsa decemlineata*, Box 2) to Finland if the climate becomes warmer and the beetle survival in Finland increases. I also investigated the potential of the insect resistant Bt-potato as an additional control strategy with eradication and field quarantine. The

invasion (establishment and spread) of the Colorado potato beetle in Finland was studied with a spatially explicit model where the landscape data consists of the occurrence of all commercial potato fields (c. 25 000) in Southern and Central Finland on a 1x1 km grid (Fig. 3).



**Figure 3.** Map of the commercial potato fields in Southern and Central Finland (reference coordinates from Information Centre of the Ministry of Agriculture and Forestry in Finland, unpublished data). The rate of the beetle invasion forms a declining gradient from south-east to the north-west and the numbers above the gradient lines tell how many beetles on average will immigrate at each cell as a Poisson process every year.

Each cell in the landscape may represent one of the four habitat types: conventional potato, Bt-potato, field in quarantine, or an empty habitat patch (matrix). Only those habitat cells that contain conventional potato are viable habitat for the beetle. However, the beetles that end up on fields with Bt-potato may survive with 5 % probability but are unable to reproduce (Nault 2001). Pest resistance in Bt potato has been achieved via the transferring of *Bacillus thuringiensis* (Bt) subsp. *tenebrions* Cry3 (used against Coleoptera) toxin gene to the plants (Shelton *et al.* 2002). The beetles were modelled as independent actors and their life cycle and the control actions against the establishment of a population are presented in the Figure 4.



(Keane & Crawley 2002). In Chapter I I investigated what life-history traits enable invasion of an intruder (e.g. GMO) into a natural species' or conventional cultivar's population. In other words, what traits could make the intruder a better competitor against the resident and lead to competitive exclusion of the resident population or coexistence of the two species?

My application of the traditional invasion analysis supported previous results by showing that invasion was possible when the intruder had a higher carrying capacity, effectively indicating a more efficient competitor of limiting resources (Tilman 1982). Although, crops (including GMOs) that have a long history in human cultivation are rarely able to persist in feral populations even though their fitness is increased by genetic modification, there may be other organisms than crops that do not have as long a domestication history and still have an ability to survive without human intervention. These include pasture plants, where the increased fitness gained from a transgene may lead to invasiveness (Godfree *et al.* 2004).

However, the main finding of the study came from the invasion analysis, when the invader was trading-off fitness traits under a common set of constraints with the resident population. The results showed that an open and unstable habitat, where competition is mainly symmetric (for example for nutrients, Weiner 1990) is prone to be invaded by a species with stable population dynamics, possibly leading to competitive exclusion of the resident population. Successful invasion and possibly competitive exclusion may result, for instance, in a plant population when the resident population's growth is mainly limited by nutrients rather than light.

Results also showed that an unstable resident population will always be outcompeted by a successful intruder and coexistence of the two competing populations is possible only when the resident population has stable population dynamics. This result supports the previous studies where disturbed sites were found to be more prone to the establishment of invading species (Grime 1973, Crawley 1986, Vilà & Weiner 2004). I also suggest that the competitive exclusion of a resident species may be more probable under disturbed than stable environments.

My findings from this study emphasize that knowledge of the traits that increase the competitive ability of the invading species or populations may not be sufficient for making reliable predictions of the invasion risk. It would be important to acknowledge that the population dynamics of both resident and intruder have an effect on the invasion success and the outcome of successful invasion which is examined in traditional invasion analysis. However, as Crawley (1997) pointed out, competitive ability of a species is also dependent on the environmental factors and other competitors and not only the traits of the species. Further, the population dynamics of the species may vary between different environments. This makes the prediction of the invasiveness of a species and also the invasibility of the resident population even more difficult and thus it is difficult to say with certainty that the introduction of a certain transgene will make a species invasive or not.

## 4.2. Escape of growth hormone fish - a threat to natural populations through mating behaviour (II, III)

The aim of Chapters **II** and **III** was to investigate further the consequences of the escape or large scale deliberate release of a growth hormone transgenic fish to the natural population. In Chapter **II** I analysed whether different mating preferences for the genotypes affect the invasion probability of the transgene. Earlier models predict that mating preference for transgenic genotypes with large size but low offspring viability will lead to the invasion of a new genotype and to a decrease in population density or even to population extinction (aka. the Trojan gene effect) as discovered by Muir and Howard (1999, 2001) with Japanese medaka fish. However, I did not record any total population extinctions, a result that contradicts Muir and Howard's (1999, 2001) findings. This was an important result, emphasizing the importance of density dependence for the invasion outcome: lower viability of transgenic fish was balanced by the decreased number of competitors, reducing the effects of density dependence. However, I did find that the equilibrium population density may decrease as a consequence of a transgenic genotype invading a natural population. Population growth rate is positive below this new carrying capacity, but the consequence of a decreased equilibrium density is an increased risk of extinction by demographic stochasticity or disturbance. Also, when population dynamics are unstable (cyclic or chaotic), the probability of extinction will increase as a result of decreased carrying capacity, because the population will frequently be subject to the risks involved with deterministically driven small population sizes (e.g., Lande 1993).

Another main finding of the study was that both different mating preference between genotypes and the initial invasion density form clear invasion thresholds. On one side of the mating preference threshold, invasion of growth enhanced fish is not possible and on the other side it can be initiated with an arbitrarily small initial density. The initial density threshold increases gradually along a gradient of mating preference however, the threshold is absent, or less abrupt, when transgenics are the most preferred genotype.

I also found that the hybrid fitness is an important determinant of whether an initially rare invader exceeds the invasion threshold, an analogous finding to that seen in pest control from the introduction of a sterility allele (Foster et al. 1972, Hedrick 2005). With low fitness of hybrids, the transgenic trait will not persist in the population, unless the introduction of transgenic fish is large enough to allow frequent mating between transgenic individuals. However, if the hybrid genotype has the highest fitness in the population (hybrid vigour), there is no threshold density for invasion and even a single individual of the transgenic or hybrid genotype may result in the invasion and persistence of the transgenic trait into the population.

In Chapter **III** I investigated how the alternative male mating strategies in Atlantic salmon could affect transgene introgression into the wild population. The main motivation to investigate this question further was the difference between the inheritance of farmed traits vs. the transgene. Hybrids resulting from the interbreeding of wild and farmed individuals usually shows intermediate traits of the parent types (Einum & Fleming 1997) but the transgene has been shown to be expressed as a single allele, meaning that it is dominant (Fletcher *et al.* 2004).



With the simulation model that combines population dynamics with the Mendelian inheritance of a transgenic trait, I found that the introgression of a growth enhancement transgene into a wild salmon population is determined mainly by how much the transgenic individuals are preferred in mating. However, the mating success of alternative male mating strategies defines how much the transgenic individuals have to be preferred in order to invade: introgression of a transgene required lower a preference of transgenic genotypes when the transgenic mature parr mating success was high in relation to anadromous males in the population. Mature parr may thus act as a vector in transgene introgression if their mating success is high. Our results are thus in line with Garant *et al.*'s (2003) findings that farmed mature parr mating advantage could speed the introgression of farmed traits (genes) into a natural population when mature parr of farmed origin have better mating success than wild mature parr, although farmed anadromous males are poor in reproduction.

I also found that the introgression of a transgene may lead to population decline and the displacement of the wild genotype by the transgenic genotype but, as in Chapter II and opposite to previous findings of transgene invasion (Muir & Howard 1999, 2001), it did not lead to population extinctions. However, population decline and the displacement of the wild genotype by a transgenic are both important conservation issues and they should both be considered as hazards in the risk assessment of transgenic fish release or escape to the wild.

Chapters II and III both emphasize the importance of detailed knowledge of the species specific reproductive systems and the effect of the transgene on both phenotype and behaviour, for the reliability of an invasion analysis. These factors are also essential to know when predicting the consequences of the escaped or deliberately introduced fish from aquaculture to the wild.

#### **4.3. Invasion of an exotic pest, Colorado potato beetle - GMO as a potential control strategy (IV)**

Within Chapter IV I turn from the negative consequences of the GMOs' to their possible benefits in the prevention of the establishment and spread of the invading exotic species, Colorado potato beetle, which is the most serious pest of cultivated potato worldwide (Crowson 1981, Hare 1990). The Colorado potato beetle is currently spreading northwards and towards Finland in Europe where its distribution is restricted by the cold climate and especially the short growing season (Sutherst *et al.* 1995, Heikkilä & Peltola 2007). The beetle has not yet established a permanent population in Finland but there is annual immigration from the source populations in Baltic and Russia (Evira 2008).

The results of my study show that the efficiency of the beetle eradication from infected fields is the most important management action for successful control supporting the previous conclusion of the importance of pre-controll of invasive pests (Convention on Biological Diversity). As long as detection and eradication methods are efficient, no population outbreaks are expected to occur. Even if eradication sometimes fails, a one year quarantine, which is a common practice in Finland after detected beetle infection, is

sufficient to prevent the establishment in current climatic conditions. In addition, the scarcity of potato fields in Southeast Finland inhibits invasion to West Finland, where potato fields form a more connected network. However, the results predict that global warming and increased beetle immigration to Finland, together with greater survival and reproduction rate, render existing control methods insufficient. If the growing season elongates due to global warming, longer field quarantine times will be needed to prevent the permanent establishment of the Colorado potato beetle population in Finland.

Control of the beetle is demanding because it has an ability to develop resistance to insecticides very rapidly and it has no natural enemies in Finland (Tauber & Tauber 2002). An option for long field quarantine would be planting Bt-potato instead of conventional cultivar, which seems to be an effective strategy. Bt potato is considered as almost non-viable (survival probability = 5 % and no reproduction) habitat for the beetle in the model and its effectiveness as a control strategy is mainly due to increased isolation between conventional potato fields which serve as viable habitat (see Fig. 4 for parameterisation). However, the benefit of the Bt potato may not be long-lasting since the Colorado potato beetle has already been shown to develop resistance against Bt when exposed to Bt sprays in laboratory studies (Ferré & Van Rie 2002). This result suggests that the evolution of resistance against transgenic Bt potato in a Colorado potato beetle population would be possible and even probable in the short term. This is one of the main reasons, in addition to marketing issues like contamination of the conventional potato varieties by genetically modified potatoes, why the genetically modified Bt-potato is not currently in commercial use (Boiteau 2005). The next step, which is already under development, is to consider the development of the resistance to Bt-potato within the CPB population.

This spatially realistic simulation model could be used as a tool to predict the future invasion potential of the CPB in cold temperature regions on a landscape level. Although we have detected the life history variables that are likely to affect the beetles' invasiveness within this study, the predictive power of the model could be improved by superimposing the model with actual climate and phenology data of the Colorado potato beetle. However, detailed life history data of the beetle in Finland is lacking and thus we decided to simplify the effect of climate change with the introduction of a dispersal gradient and by varying beetle life history parameters.

## 5. Conclusions

The main theme of this thesis was to study ecological invasion in relation to genetically modified organisms, and what kind of consequences invasion may have in natural populations. These themes concentrate on hazard identification part of ecological risks assessment of genetically modified organisms. According to the results of this thesis the concerns raised about the large scale use of genetically modified organisms in agri- and aquaculture are well justified: we found invasion of a transgenic species or genotypes to be possible under certain realistic conditions and resulting in competitive exclusion (**I**), population decline through outbreeding depression (**II**, **III**) and genotypic displacement of native species (**II**, **III**). However, we also found that previous predictive models may have

given too pessimistic a view of some case studies. For instance, escape of the growth hormone transgenic fish to a natural population may not necessarily lead to total population extinction (II) as previously suggested (Muir & Howard 1999, 2001). These results bring valuable new information which can be applied to the risk assessment of genetically modified organisms.

It has been acknowledged that the risk assessment of genetically modified organisms should be made in comparison to their conventional alternatives and their benefits should also be included in the analysis (cost-benefit analysis) (Suter 1993). In the last part of this thesis I found that GM potato could serve as an option in preventing the establishment of a serious exotic pest of potato, Colorado potato beetle, in Finland (IV). The additional benefits of a GM variety would be the reduction of insecticide use and reduced need for field quarantine, which reduces the economic losses unable to cultivate potato on certain fields in certain years.

Different life history traits of the species have been proposed to serve as predictors of invasiveness in studies of many species and also when assessing the increased invasiveness of genetically modified organisms (e.g., Baker 1974, Mollison 1986, Rejmánek 1996, Kolar & Lodge 2001, Rejmánek *et al.* 2003, Crawley *et al.* 1993). The results of Chapter I emphasise that the traits of the invading species may not solely determine its invasion ability to the resident population, but factors affecting population dynamics of both invading and the resident species' also have to be taken into account in risk assessment. Habitat disturbance has been shown to benefit the invading populations (Higgins & Richardson 1996, Freckleton & Watkinson 1998, Vilà & Weiner 2004) but in Chapter I I showed that population fluctuations caused by intrinsic demographic factors leading to unstable population dynamics might also be an important determinant of invasibility of the population.

In the assessment of the invasion risk of GM plants the key issue is to assess if the transgene increases or differentiates the weedy characteristic or traits of the organism (Conner *et al.* 2003). Transfer of, for example, a cold or salt tolerance gene to a plant that is already known to be invasive in other environments, could create a genotype that could invade habitats where the unmodified genotype can not. Results of this thesis suggest that modifications that increases the species competitive ability for, e.g., light or territories (asymmetric competition), may produce a competitively superior invader and the invasion may lead in the worst case to competitive exclusion of the resident population (I).

Previous studies have suggested that the invasion history of the species is a good predictor of its invasiveness: if the species is known to be invasive in some parts of the world it is probably invasive in other regions too (Kolar & Lodge 2001, Conner *et al.* 2003). If the species is known to have invasive history and extensive adaptability to varying environmental conditions, as is the case in the Colorado potato beetle, it might be crucial to determine what kind of changes in life history traits may lead to the invasion in new areas (IV). This should also be kept in mind when predicting the probability of increased invasiveness of genetically modified organisms.

Although there is as low probability that GM crops will become invasive or even persist in the wild due to effects of the introduced traits, they may have compatible relatives to hybridize with. To assess the possible risks of escaped transgenic animals may

be more complicated than assessing the effects of GM crops. Mating behaviour and sexual selection play important roles in animal populations in defining what genotypes succeed to reproduce and pass their genes to the next generation and thus lead to spread of the transgene. My results (II, III) supported the previous findings that if, for example, growth hormone transgene has a male mating advantage and a general viability disadvantage, conditions for invasion to the natural population remain very broad (Hedrick 2001). Even though I did not record any population extinctions, the displacement of the wild genotype by an artificial one should be considered as serious a consequence as total population extinction in risk assessment. It will thus be crucial to take into account different kinds of behavioural differences among species when assessing the possible hazards that GMOs may cause if they escape to the natural population.

It has been suggested that integrating population biological and evolutionary modelling with empirical data could be a promising way for estimating probabilities of ecological scenarios (like risk assessment) (Damgaard & Lokke 2001). Models presented in this thesis could be used as tools to predict the long term consequences, both negative and positive, of GMOs in natural populations and cultivated environments. To apply these models to a case specific risk assessment, they may be supplemented with detailed data of the species biology, the effect of the transgene introduced to the species, and also the characteristics of the populations or the environments in the risk of being invaded.

## 6. Acknowledgements

First of all, I would like to thank my supervisors Veijo Kaitala and Sami Aikio for giving me an opportunity to come in on a project of a very current and hot topic of GMOs and their ecological risks. I am truly grateful for all your support during the journey, especially, the stressful last phases of this project. Sami, I would like to thank you for always having time and patience for my endless questions and also for being such a good friend! I also want to thank Esa Ranta for providing the facilities for working in IKP and for collaboration.

I also want to thank other collaborators who have contributed on this work: Per Lundberg, Johanna Mappes and Leena Lindström. Jonna and Leena, you provided me the first hand knowledge of the Colorado potato beetle life history in Finland, which was essential, and also Anne and Saija, I really enjoyed our discussions on the matter during conferences in Vienna and Turku. I wish to thank all ESGEMO people for fruitful discussions and pleasant times during our annual meetings. John Reynolds and Bob O'Hara pre-examined this thesis and their constructive comments were highly valuable. I also want to express my gratitude to Mike Fowler for revising the language of the thesis and Jouni Laakso for comments on the early version of the summary.

IKP people, it has been a pleasure to work with all of you! I would like to thank all the roomies I've had during the four years, while our "girls' office" have slowly changed to the "boys' office". Especially I want to thank "IKP Girls", Annu, Ansku, Hanna K, Katja, Katriina, Nina, and Tiina, for hilarious times in and out of office.

The Department has been a pleasant environment to work in. I would especially like to acknowledge Jonna Katajisto and Hannu Pietiäinen for your trust on this work. It means a lot to me. Ilkka Teräs and ladies from the fourth flour; I have always been able to rely on your help on numerous bureaucracy questions. Thank you!

For the research period preceding this thesis, I want to thank Marko Nieminen and Ilkka Hanski for giving me an opportunity to spend several sunny summers on the beautiful Åland Islands. Those days made me an ecologist. Hanna P, a woman could not have a better companion on the fieldwork than you! Thanks for being such a good friend!

I would also like to thank my new colleagues at the Academy of Finland for your understanding and support while I have been chasing between the final phases of this project and my new responsibilities. I'll promise to give my full investment to Academy duties hence!

My dear old friends! Heini, Mari, Pate, Päivi, Susa; I've been exceptionally lucky to have you all as my friends for so long time, longer than not! Moreover, I want to thank all the extended "Itis-Vartsika jengi" for the great times we've shared together. I'm looking forward to new adventures with you. Majka, thank you for the beautiful cover design of this thesis, and also for bearing out with me during the last hectic months of this project. I also want to thank Outi for your great friendship and for a lovely foster child!

Last, I would like to thank my family. Äiti ja isä, kiitos että olette olleet tukenani ja arvostaneet valintojani. Se on merkinnyt minulle paljon! Make, kiitos rakkaudestasi ja kärsivällisyydestäsi ja että pidät jalkani maassa. Ilman sinua maailmani olisi paljon tylsempi paikka.

Information Centre of the Ministry of Agriculture and Forestry in Finland provided the data of potato fields in commercial cultivation in Southern Finland. This research was funded by the Academy of Finland, Ministry of Agriculture and Forestry and Ministry of the Environment in Finland as a part of research programme on Environmental, Societal and Health Effects of Genetically Modified Organisms (ESGEMO), and Chancellor of the University of Helsinki.

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