Population differentiation in sticklebacks: disentangling maternal, environmental and genetic effects

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Faculty of Biological and Environmental Sciences
University of Helsinki 2013

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Academic dissertation

To be presented for public examination with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki in Latokartanonkaari 5 - C-rakennus, Luentosali 1 (K103), on 7th February 2014 at 12 a.m.

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Cover design by: Izza
ISBN 978-952-10-9690-7 (paperback)
ISBN 978-952-10-9691-4 (PDF)
http://ethesis.helsinki.fi
Unigrafia, Helsinki 2013

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This thesis is based on the following articles, which are referred to in the text by their Roman numerals:

I. **Ab Ghani N I, Herczeg G & Merilä J. 2012.**
Body size divergence in nine-spined sticklebacks: disentangling additive genetic and maternal effects. *Biological Journal of the Linnean Society*, 107, 521–528.

II. **Ab Ghani N I, Herczeg G, Leinonen T & Merilä J. 2013.**
Evidence for genetic differentiation in timing of maturation among nine-spined stickleback populations. *Journal of Evolutionary Biology*, 26, 775–782.

III. **Herczeg G, Ab Ghani N I & Merilä J. 2013.**
Evolution of stickleback feeding activity: genetics of population divergence at different ontogenetic stages. *Journal of Evolutionary Biology*, 26, 955–962.

IV. **Ab Ghani N I, Kuparinen A, Leinonen T & Merilä J. 2013.**
Population and sex-specific divergence in growth patterns between two nine-spined stickleback (*Pungitius pungitius* L) populations. *Evolutionary Ecology Research*, in press.

V. **Ab Ghani N I & Merilä J. 2013.**
Population divergence in compensatory growth responses and their costs in sticklebacks. *Manuscript.*

VI. **Ab Ghani N I & Merilä J. 2013.**
Effects of compensatory growth on female reproductive and offspring traits in nine-spined sticklebacks. *Manuscript.*

Author contribution to the articles

	I	II	III	IV	V	VI
Concept	NIAG, GH, JM	NIAG, GH, JM	GH, NIAG,	NIAG, AK, JM	NIAG, JM	NIAG, JM
Data collection	NIAG	NIAG	NIAG	NIAG	NIAG	NIAG
Analyses	NIAG, GH	NIAG, GH	GH	NIAG, AK	NIAG, JM	NIAG, JM
Manuscript preparation	NIAG, GH, JM	NIAG, GH, TL, JM	GH, NIAG, JM	NIAG, AK, TL, JM	NIAG, JM	NIAG, JM

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Abstract

Isolated populations of fish living in ponds are often phenotypically and genetically diverged from their source populations. However, to what degree this divergence reflects maternal, genetic or environmental effects is often unclear, as is the possible adaptive nature of this differentiation due to the strong impact of random genetic drift on populations residing in isolated habitats.

The aim of my thesis was to investigate the relative influence of genetic, environmental and maternal effects on phenotypic differentiation using Fennoscandian populations of nine-spined sticklebacks, *Pungitius pungitius*, as a model system. I used controlled crosses of fish from an ancestral Baltic Sea and a derived Pyöreälampi pond population – known to differ both genetically in neutral marker genes (F_{st} = 0.46) and phenotypically in numerous traits (*viz.* morphology, anatomy, behaviour and life history) – to explore the relative importance of additive genetic, non-additive genetic, environmental and maternal effects as determinants of population differentiation in morphology, life history, and behaviour.

I found evidence for strong genetic contributions to divergence in all studied traits (*viz.* body size, growth, age at maturity, feeding behaviour), but these genetic effects were not always additive. Rather, strong non-additive (dominance) contributions were observed in some traits, such as age at maturation. Furthermore, evidence for age-dependent dominance was found in the case of feeding behaviour. These results indicate that much of the phenotypic differentiation among marine and pond populations of nine-spined sticklebacks is due to genetic, rather than to environmental or maternal effects. Nevertheless, results from feeding manipulation experiments revealed a great deal of phenotypic plasticity in growth rates and patterns, and in particular, clear evidence for recovery growth. Specifically, full recovery – and even over compensation – was observed in response to unrestricted feeding following a period of resource limitation. The results of these experiments also indicate that there are genetically based population differences in recovery growth potential, and that maternal effects play into explaining patterns of recovery growth in response to feeding treatments. Furthermore, the impact of feeding treatments experienced by mothers was found to carry-over to influence the phenotypes of their offspring.

Taken together, the results presented in this thesis demonstrate that the observed phenotypic differences among marine and pond populations of nine-spined sticklebacks are likely to be based on genetic differentiation, although the mode of gene action underlying this differentiation is not always additive. Although environmental and maternal effects were shown to be important modulators of phenotypic variation in this system, their role in explaining population differentiation appears to be secondary. Further studies utilizing an F_2-backcross-design – as well as replicate populations – might shed more light on the questions that remain open. Nevertheless, these results reinforce the conjecture that Fennoscandian pond populations of nine-spined sticklebacks might, or should be viewed as a significant evolutionary and conservation unit that require special attention in regional and national management and conservation plans.

Introduction

When individuals disperse from their ancestral population to colonise new environments, they are faced with challenges that differ from those in their native environment. Most individuals probably fail to overcome these challenges, but those few that do become the founders of a new population. Over time, this new population may diverge from the ancestral population, through three processes: (i) natural selection, which leads to local adaptation (e.g. Kawecki & Ebert 2004; Blanquart et al. 2013), (ii) random genetic drift, which is a consequence of non-random sampling of the genetic variation in the founder population (e.g. Lande 1976; Barton 1996) or (iii) phenotypic plasticity, which may reflect either a neutral (e.g. stress) or adaptive response to new environmental conditions (e.g. Gotthard & Nylin 1995; West-Eberhard 2003; Ghalambor et al. 2007).

One important distinction among these different and mutually non-exclusive explanatory models for population differentiation is that the two first explanations both require a genetic basis for the observed divergence, whereas the third explanation by definition postulates an environmental basis for the observed divergence. While a number of different approaches have been developed to differentiate between natural selection and genetic drift as causes of population differentiation in a given phenotypic trait (e.g. Ovaskainen et al. 2011; Leinonen et al. 2013), most of these approaches require either assumptions regarding, or some degree of understanding of, the genetic basis of observed trait differentiation. In other words, whether caused by natural selection or genetic drift, the requisite for postulating an evolutionary explanation (as opposed to simple phenotypic plasticity) for population differentiation is that the difference has a genetic basis – evolution is by definition a change in the genetic constitution of population.

In theory, genetic divergence among populations can be attributed to additive genetic, non-additive genetic, and maternal effects – the latter itself having both genetic and environmental components (Falconer & Mackay 1996). Traditionally, non-additive genetic effects have often been overlooked because they are difficult to estimate with the sample sizes usually available for experimentalists, and because evolutionary biologists are usually mostly interested on additive genetic effects (Lynch & Walsh 1998). However, there is increasing evidence to indicate that non-additive genetic effects, such as dominance and epistasis, can be important sources of variation explaining population differentiation in quantitative traits (Wolf et al. 2000; Phillips 2008).

Studies on the genetic architecture of phenotypic traits have also revealed that the relative contributions of additive genetic, non-additive genetic and maternal effects can change during development (Falconer & Mackay 1996; Lindholm et al. 2006; Shimada et al. 2011). Typically, the expression of phenotypic traits during early life stages tends to be strongly influenced by maternal environmental effects, whilst the importance of additive and non-additive genetic effects increases at later stages (Falconer & Mackay 1996; Lindholm et al. 2006; Shimada et al. 2011). Likewise, variation in life-history traits is often strongly correlated with variation in fitness, and life-history traits tend to express more non-additive

genetic variance than morphological traits (Houle et al. 1996; Merilä & Sheldon 1999). Less is known about this with respect to behavioural traits, which are also often strongly correlated with fitness proxies, and for which additive genetic (e.g. Dingemanse et al. 2002, 2009; Drent et al. 2003; van Oers et al. 2004) as well as non-additive genetic and maternal effects have been reported (e.g. Dingemanse et al. 2012). Therefore, further studies aiming to disentangle additive genetic effects from non-additive and maternal effects are needed, particularly in the context of studying adaptive divergence and population differentiation in phenotypic traits.

Environmental effects can also interact with genetic effects to generate both non-adaptive and adaptive plasticity (Gotthard & Nylin 1995; Hutchings 2004). Such genotype-by-environment interactions (G × E interactions) are a common form of phenotypic plasticity (Kawecki & Ebert 2004; Whitman & Agrawal 2009) and neatly described with reaction norms (Schlichting & Pigliucci 1998). The occurrence of phenotypic plasticity can be restricted to a given generation or developmental stage, but can also persist over generations, a phenomenon known as cross-generational phenotypic plasticity (Rossiter 1996; Donohue & Schmitt 1998; Mousseau & Fox 1998; West-Eberhard 2003, 2005; Salinas & Munch 2012). When occurring, cross-generational phenotypic plasticity refers to ways in which parents alter the traits of their offspring in response to environmental conditions, and hence, are also known as environmental maternal effects (Solemdal 1997; Donohue & Schmitt 1998; Mousseau & Fox 1998; Salinas & Munch 2012).

Phenotypic plasticity, including environmental maternal effects, can be adaptive and beneficial (Donohue & Schmitt 1998; Mousseau & Fox 1998; Laurila et al. 2002; Allen et al. 2008). However, phenotypic plasticity can also be a passive stress response and even maladaptive (Ghalambor et al. 2007). This suggests that being phenotypically plastic entails a cost. The cost of phenotypic plasticity can be defined as the extra costs (loss of fitness) that an individual has to pay in expense of expressing a plastic phenotype in comparison to having a fixed phenotype (de Witt 1998; Pigliucci 2005). The cost can be paid either in the current (de Witt et al. 1998) or in subsequent generations (e.g. Donohue & Schmitt 1998; Bashey 2006).

It is, thus, evident that some knowledge of the genetic mechanisms underlying trait variation (i.e. additive, non-additive and maternal effects) is required in order to understand the ultimate and proximate causes of population differentiation. However, environmental effects (including G × E interactions) may also play a role in shaping the traits of interest. Disentangling genetic *vs.* environmental differentiation between divergent populations is traditionally studied with the aid of common-garden or reciprocal transplant experiments (e.g. Kawecki & Ebert 1994; Blanquart et al. 2013; Savolainen et al. 2013). While common garden and reciprocal transplant experiments can help to better understand the relative contributions of gross-scale genetic and environmental effects on trait variation, they do not as such provide much insight into the genetic architecture of the divergence. Moreover, they may sometimes confound maternal and cross-generational environmental effects with genetic effects (e.g. Kaplan 1998; Laugen et al. 2002). However, common

garden experiments coupled with appropriate mating designs provide one way to gain insights into most of these issues. Two kinds of common garden approaches can be used: (i) offspring from different populations are reared under common environmental conditions for a few generations to eliminate cross-generational environmental influences, but this approach may not be possible for animals with long generation times (Rossiter 1996; Lacey 1998). Alternatively, (ii) reciprocal crosses between different populations can be performed, and the offspring reared in common garden settings to compare trait values in the different hybrid and pure crosses (e.g. Azevedo et al. 1997; Laugen et al. 2002). In this thesis, the latter approach was chosen. This approach allows also probing into the relative importance of genetic (both additive and non-additive) and maternal effects, and also, to some extent, identification of patterns owing to simple dominance (Azevedo et al. 1997; Laugen et al. 2002). More complicated and refined back-cross designs allowing partitioning of variance in traits of interest to various causal components are also possible (e.g. Lynch & Walsh 1998), but were not used due to logistical constraints (see below).

The study system

The nine-spined stickleback (*Pungitius pungitius*) is a small teleost fish, belonging to the *Gasterosteidae* family, this fish is widely distributed mainly in brackish and freshwater habitats throughout the northern hemisphere (Öslund-Nilsson et al. 2007). It is also found in the marine habitat, where it lives in sympatry with a number of piscine predators, such as salmonids (*Salmo* spp.), perch (*Perca fluviatilis)*, pike (*Esox lucius)*, and pikeperch (*Sander lucioperca)*. It sometimes also co-occurs with its interspecific competitor, the three-spined stickleback (*Gasterosteus aculeatus)*, but in many freshwater localities – especially in isolated ponds in Fennoscandia – nine-spined sticklebacks occur in the absence of both piscine predators and interspecific competitors.

Marine and pond populations of nine-spined stickleback have been reported to show repeated phenotypic divergence in numerous morphological, behavioural and life history traits (reviewed in: Merilä 2013; Box 1). This divergence has taken place during the past 8000 years, after the colonization of this formerly glaciated area (Eronen et al. 2001). The presumed ancestral marine form that has fed the colonisations is small-sized (usually < 5 cm in standard length) with adult weight around 1 – 2 g (Herczeg et al. 2009c), and produces small clutches (Herczeg et al. 2010c). They exhibit high growth rates and early maturation (Shimada et al. 2011). Behaviourally, marine nine-spined sticklebacks are inactive feeders, shy, non-aggressive and risk-averse (Herczeg et al. 2009a, b; Herczeg & Välimäki 2011). They also exhibit well-developed body armour (Herczeg et al. 2010b), large *bulbus olfactorii* and *telencephala* (Gonda 2011), and a low number of neuromasts (Trokovic et al. 2011; Välimäki 2012). The descendent pond populations, which in contrast to their marine congeners live in the absence of piscine predators and interspecific competitors, are 'giant'-sized (usually ca. 8 – 10 cm in standard length, sometimes > 11 cm; Herczeg et al. 2009c, 2010a; Merilä 2006) with adult weight more than 8 g (Herczeg et al. 2009c; Fig. 1). They produce clutches which are two to three times larger than marine fish (Herczeg et al. 2010a). Likewise, presumably due to the absence of piscine predation

pressure, the pond nine-spined sticklebacks have reduced body armour (Herczeg et al. 2009a, 2010b). In contrast to their marine conspecifics, they have small *bulbus olfactorii* and *telencephala* (Gonda 2011), and a high number of neuromasts (Trokovic et al. 2011; Välimäki 2012). The pond nine spined sticklebacks are also long-lived, reaching ages up to at least seven years, and they appear to mature earliest at the age of two to three years (Herczeg et al. 2009c; Shimada et al. 2011). Behaviourally, pond nine-spined sticklebacks are active feeders, bold, and aggressive risk takers (Herczeg et al. 2009a,b).

Fig. 1. The two forms of Fennoscandian nine-spined sticklebacks (*Pungitius pungitius*) used in this thesis. The fish on the right is a male from the ancestral Baltic Sea stock, whilst the fish on the left is a female of the descendent Pyöreälampi pond stock. Photo courtesy of Chris Eberlein.

Box 1. Fennoscandian nine-spined sticklebacks

Fennoscandian nine-spined sticklebacks from the Baltic Sea and Pyöreälampi are well-suited models to study adaptive divergence. Pond and marine populations, these two included, have been reported to show repeated phenotypic divergence in numerous morphological, behavioural and life history traits (reviewed in: Merilä 2013). The following table details some of the morphological, behavioural and life history trait differences which are consistent between marine and pond nine-spined stickleback populations.

Phenotypic trait	**Marine**	**Pond**
Body size of adult fish	Normal, small-sized (usually < 5 cm in standard length)	'Giant'-sized (up to 12 cm in standard length)
Body weight of adult fish	1 – 2g	≥ 8g
Body armor and defense structures	Well-developed	Reduced
Brain	Large *bulbus olfactorius* and *telencephalon*	Small *bulbus olfactorius* and *telencephalon*
Number of neuromasts	Low	High
Growth	High growth rate and short growth period	Slow growth rate and extended growth period
Timing of maturation	Early, i.e. maturing a year after hatching	Late, i.e. maturing two to three years after hatching
Life span	One to two years	Can live up to seven years (perhaps longer)
Behaviour	Inactive feeder, shy, non-aggressive and risk-averse	Active feeder, bold, aggressive and risk taker

Previous studies on phenotypic differentiation in morphological (e.g. Herczeg et al. 2009c, 2011; Shikano & Herczeg et al. 2010a, 2012; Shimada et al. 2011; Aikio et al. 2013) between the marine and pond populations suggest that the observed differences have a genetic basis. However, since all of these studies are based on rearing of first generation full-sib families in common-garden experiments, the possible contributions of non-additive genetic effects, plasticity (G × E interactions) and maternal effects on this divergence have remained uncertain.

Aims of this thesis

The overall objective of this thesis was to assess the relative roles of genetic, environmental and maternal effects (either genetic or environmental) as proximate mechanisms underlying phenotypic divergence between the putative ancestral marine and derived pond populations of nine-spined sticklebacks. More specifically, the aim was to disentangle additive genetic effects from non-additive genetic (dominance) and maternal effects, and to assess the influence of genotype-environment interactions (phenotypic plasticity) on population differentiation. To this end, I focussed on studying development and divergence in different types of traits including morphological (body size), life history (timing of maturation, growth rate, female reproductive investment and early larval traits), and behavioural (feeding activity) traits in these two populations and their reciprocal hybrids.

In Chapters **I**, **II**, **III** and **IV**, my aim was to evaluate the relative importance of additive and non-additive genetic effects, as well as the role of maternal and environmental effects on phenotypic divergence between Baltic Sea and Pyöreälampi pond populations. Here, I investigated morphological (body size; Chapter **I**), life history (timing of maturation and growth; Chapters **II** & **IV**) and behavioural (feeding activity; Chapter **III**) traits in a common garden experiment. In Chapters **II** and **IV**, my aim was also to explore the potential influence of sex on timing of maturation and growth since earlier studies (Shimada et al. 2011; Herczeg et al. 2012) suggest differences between sexes in these traits. In Chapter **IV**, I also aimed to find out whether timing of maturation can influence growth, and conversely, whether there are indications that growth (rates) can affect timing of maturation.

In Chapter **V,** my aim was to look for the existence and magnitude of compensatory growth (Box 2) in nine-spined sticklebacks, and to explore the possible costs of such responses in terms of an individual's intrinsic survival and maturation probabilities. In addition, I also investigated the relative influences of additive and non-additive genetic effects, as well as maternal effects, in these responses. In order to accomplish these goals, I used experimental manipulation of feeding regimes to test for growth responses of sticklebacks originating from different intra- and inter-population crosses.

The chapter **VI** is a follow up of chapter **V**, with the aim to determine whether and how environmental conditions experienced during an individual's development influence their reproductive output and the phenotypic characteristics of their offspring. In other words, I was interested to see if investment in compensatory growth traded-off with individual fecundity, and if investment in compensatory growth in females traded-off with their

Box 2. Growth patterns in fish

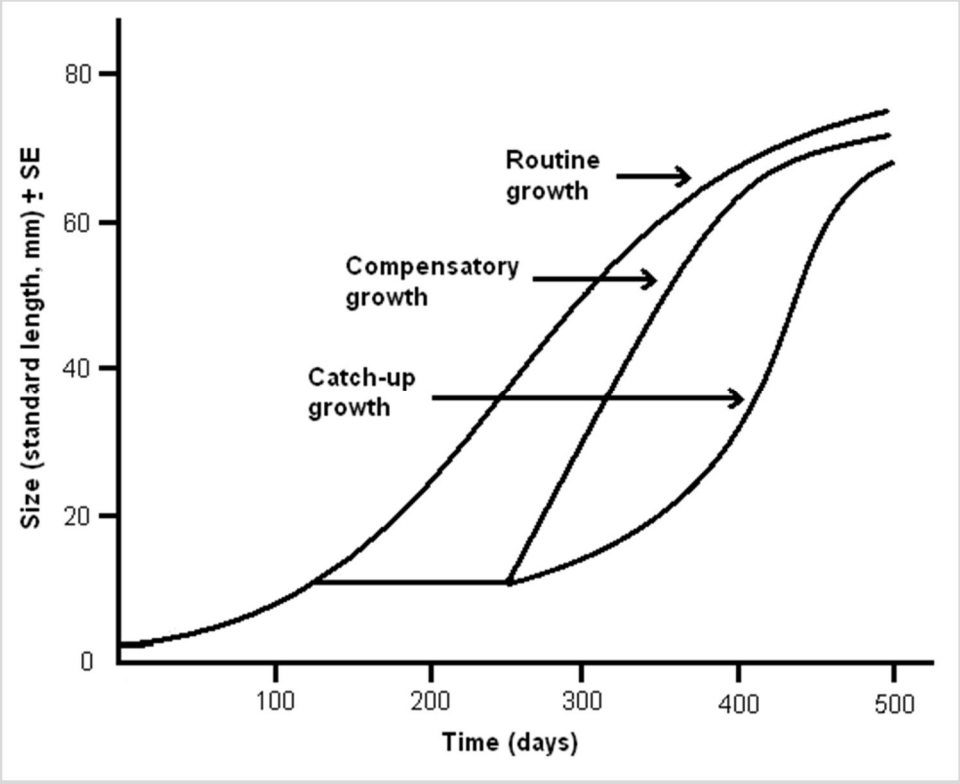
There is a fair bit of variation, even confusion, surrounding the terminology used to define growth patterns in fish. The following definitions are followed in this thesis:

Routine growth refers to a normal pattern of growth. It is characterized by rapid increase in size-at-age in the beginning, followed by diminishing growth increments later on. Graphically, it can be illustrated as a sigmoidal growth-curve (see enclosed figure).

Compensatory growth occurs when favourable conditions are restored after a period of growth depression, and growth accelerates to catch-up to the original growth trajectory (Metcalfe & Monaghan 2001; Ali et al. 2003; Jobling 2010). An important and defining feature of compensatory growth is that it is faster than growth of similar sized individuals undergoing routine growth (Nicieza & Álvarez 2009; Jobling 2010; see enclosed figure).

Catch-up growth refers to the ability of individuals subject to a period of stagnant growth to resume normal growth when they are returned to favorable conditions (Jobling 2010). Catch-up growth can occur without growth acceleration above routine levels (see enclosed figure).

Recovery growth is a generic term that refers to either compensatory or catch-up growth situations. In general, recovery growth can take one of the following forms: (i) partial compensation in which individuals subject to food restriction do not achieve the same body size as normally/continuously fed individuals, (ii) full compensation where previously food-restricted fish reach the same body size as normally/continuously fed fish, and (iii) over compensation in which body size of the previously food-restricted fish ends up exceeding that of normally/continuously fed fish, (Ali et al. 2003; Metcalfe & Monaghan 2003).



offspring quality in the next generation. In addition, I wanted to find out if the influence of experimental treatments were independent of direct maternal influences related to reproductive investment (i.e. clutch, egg and yolk sizes).

Materials and Methods

Sampling

Results presented in this thesis are based on a common garden experiment performed between June of 2010 and January of 2012. The parental fish were collected from the wild during the early phase of the reproductive season in late of May to mid of June 2010. Two populations were sampled: a marine population from the Baltic Sea at Helsinki (60°12′09″ N, 25°10′58″ E), which was sampled using a seine net, and a pond population from Pyöreälampi pond in the Kuusamo area (66°15′40″ N, 29°26′00″ E), which was sampled using minnow traps (mesh size of 6 mm in both). These two populations are isolated both geographically (~900 km) and genetically (neutral molecular marker divergence F_{ST} = 0.46; Shikano et al. 2010; Shimada et al. 2011). The marine site is a shallow coastal, brackish (0 – 6 psu) water bay with a heterogeneous habitat where nine-spined sticklebacks live in sympatry with piscine predators such as salmonids (*Salmo* spp.), perch (*Perca fluviatilis*), pike (*Esox lucius*), pikeperch (*Sander lucioperca*), as well as with interspecific competitors such as the three-spined stickleback (*Gasterosteus aculeatus*). In contrast, Pyöreälampi is an isolated pond with a surface area of less than 5 ha. The nine-spined stickleback is the only fish species occurring there, with a possible exception of recently introduced whitefish (*Coregonus lavaretus* Linnaeus, 1758), though these may now be extirpated (J. Merilä, personal communication).

General experimental conditions

Artificial crosses were made *in vitro* between randomly chosen males and females by gently squeezing the eggs from ripe females and pipetting a sperm solution onto the eggs. The sperm solution was obtained by mincing the testicles of over-anaesthetized (with ca. 100 mg L^{-1} of MS-222) males in a drop of water. Each parent was used only once (i.e. all crosses consisted of full-sib families). Fertilized eggs (hereafter: clutches) were kept in petri dishes in filtered tap water (water changed twice a day) until hatching. Developing clutches were checked under a dissecting microscope regularly and dead or unfertilized eggs were removed.

After larvae started to swim freely (ca. 7 days after hatching), they were randomly assigned to individual 1.4-L tanks on one of four Allentown Zebrafish Rack Systems (hereafter referred to as racks; Aquaneering Inc., San Diego, CA, USA). Each rack contained 100 units of 1.4-L tanks and had a closed water circulation system equipped with physical, biological, and UV filters. Visual contact between tanks was blocked using opaque plastic panels. Fish were reared in freshwater (salinity 0 psu), and therefore osmoregulation-related issues should contribute little to differences in measured traits. Note that the parental fish from the Baltic Sea originate from very low salinity (0 – 6.0 psu; Shimada et al. 2011) waters.

Experimental designs

Three different common garden experiments were included in this thesis. In brief, for the first common garden experiment, utilized in chapters **I-IV**, four different types of first generation (F_1) crosses were produced:

two ‘pure’ crosses by crossing either Helsinki males with Helsinki females (Hel–Hel) or Pyöreälampi males with Pyöreälampi females (Pyö–Pyö), and two ‘hybrid’ crosses by crossing either Pyöreälampi males with Helsinki females (Pyö–Hel) or Helsinki males with Pyöreälampi females (Hel–Pyö). A total of 200 individuals from 10 full-sib families per cross-type (i.e. five individuals per family from each cross-type) were produced for the purposes of Chapters **I-IV**. All fish in this experiment were reared in high feeding treatment (for details on rearing conditions, see Table 1).

For the second common garden experiment, utilized in Chapter **V**, four different types of F_1 crosses were produced as described above. A total of 400 individuals consisting of 10 full-sib families per cross-type (ten individuals per family for each cross-type) were produced and reared in three different feeding treatments: high feeding (fish were fed twice a day: once during the morning and the afternoon), low feeding (fish were fed once every two days during the morning feeding), and recovery feeding (low feeding treatment for the first three months after hatching [7 – 90 DAH = days after hatch], followed by high feeding treatment afterwards [91- 510 DAH]; Chapter **V**). For details on the rearing conditions, see Table 1.

For the third common garden experiment, utilized in Chapter **VI**, a total of five full-sib families were produced (F_1) by crossing Helsinki males with Helsinki females (Hel–Hel). In total, 15 males and 15 females were reared in three different feeding treatments described above (*viz*. high, low and recovery feeding; five pair of individuals per treatment). Later, these 15 males and 15 females were crossed to produce a total of 375 offspring, i.e. the F_2 generation (25 individuals per female for each treatment), and reared thereafter in identical conditions (17°C; 24h light) until hatching. The experimental design is illustrated in Fig. 2.

The high feeding treatment employed here represents an optimal environment where body size, timing of maturation, growth and female reproductive investment are normal, and the fish are expected to develop in the absence of nutritional constrains. The low feeding treatment represents a stressful environment in which nutritional restriction is expected to result in reduced growth rates, body size, and delayed timing of maturation. The stressful environment may also have a negative impact on female reproductive investment. The recovery feeding treatment represents a shift between two treatments, allowing opportunity for a recovery in growth after the nutritional constraints are removed. Further details regarding the experimental designs and procedures can be found in the individual Chapters (**I-VI**).

Measured traits

(i) Body size

Standard length (from the tip of the lower jaw to the base of the caudal peduncle) was used as a measure of body size. It was measured from photographs (of live fish) taken with a digital camera (Nikon D60) to the closest 0.01 mm using the TPSDIG 2 (Rohlf 2002). A ruler was placed in each photograph for a size reference. At 337 DAH, 188 individuals from the high feeding treatment (Hel–Hel: 45, Hel–Pyö: 48, Pyö–Hel: 49 and Pyö–Pyö: 46; Chapter **I)** were measured. At 510 DAH, 200 individuals from three different feeding treatments: 126 individuals (Hel–Hel: 25, Hel–Pyö: 33,

Table 1. Detailed description of rearing conditions for individual Chapters (**I-VI**) included in this thesis.

Chapter	Rearing condition
I-III	Fish were kept under 14:10 h light:dark photoperiod, with water temperature at 17°C for 299 days after hatch (hereafter DAH). At 300 DAH, over a two week period, fish were put into artificial hibernation, where the photoperiod was gradually shifted to 0:24 h light:dark, and the water temperature was gradually lowered to 4°C. Artificial over-wintering lasted for 30 days. After 30 days, water temperature was gradually increased back to 17°C, and the photoperiod was gradually increased to 24h light over a two week period. All the fish were then kept at 17°C and 24h light for 97 days before the experiments were terminated.
IV-VI	Fish were maintained at 14:10 h light:dark photoperiod, with water temperature at 17°C for 299 DAH. At 300 DAH, over a two week period, fish were put into artificial hibernation (as above). After 30 days, and over a two week period, water temperature was gradually increased back to 17°C, and the photoperiod was gradually increased back to 24h light. All fish were then kept at 17°C and 24h light for 97 days. At 455 DAH, all fish were put under a second artificial hibernation, following the protocol above. After 30 days under the second artificial hibernation, photoperiod and temperature were increased (as above). All fish were kept under these conditions until 510 DAH. At 510 DAH, the experiment was terminated.

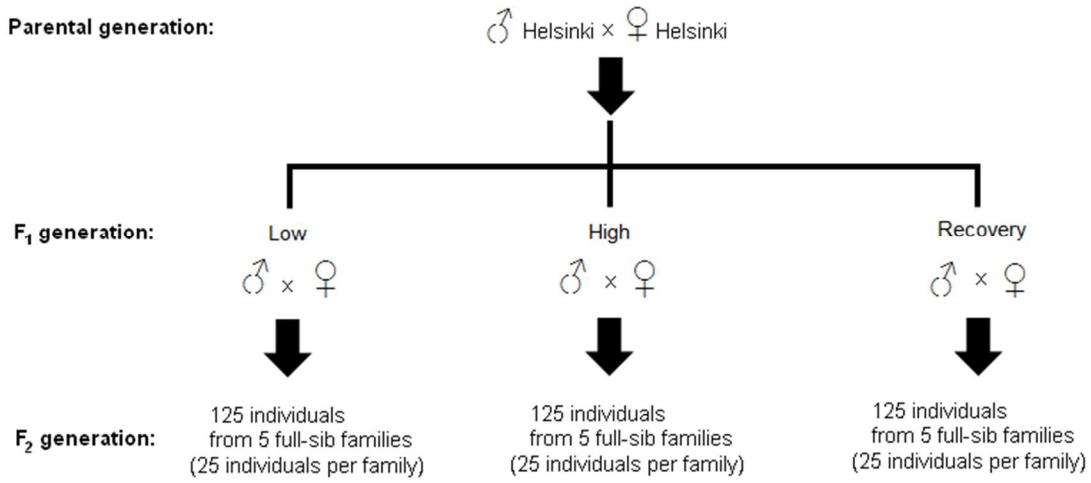


Fig. 2. A schematic illustration of the experimental design used in Chapter **VI**. Five families were formed from wild collected parents to produce the F_1-generation. Ten members of each family were subjected to each of the three feeding treatments (high, low and recovery feeding treatments, respectively) and reared to adulthood. Only one member of each family which was subjected to three feeding treatments was used to produce F_2-offspring. Twenty-five F_2-offspring per family, born to F_1-parents in different treatments, were measured to assess larval traits in the F_2-generation.

Pyö–Hel: 24 and Pyö–Pyö: 44) from high, 32 individuals (Hel–Hel: 13, Hel–Pyö: 10, Pyö–Hel: 6 and Pyö–Pyö: 3) from low, and 42 individuals (Hel–Hel: 4, Hel–Pyö: 8, Pyö–Hel: 16 and Pyö–Pyö: 14) from the recovery feeding treatment, were measured (Chapter **V**). Also, at 510 DAH, 15 females from the marine population (Hel–Hel) from three different feeding treatments (high: 5, low: 5, and recovery: 5) were measured (Chapter **VI**).

(ii) Timing of maturation

Maturation was assessed by a visual inspection of external characteristics. Sexually mature nine-spined stickleback males develop white pelvic spines and black body colouration, while sexually mature females develop a protruding belly as a consequence of the ovaries containing ripe eggs. Sex of immature individuals at the end of the experiment was identified using a molecular method following Shikano et al. (2011). In short, DNA was extracted from a small portion of caudal fins of the immature individuals using the chelex method (Walsh et al. 1991). Polymerase chain reactions (PCRs) were carried out with a sex-linked microsatellite marker Stn19 (Shikano et al. 2011). The PCR methods are described in Chapter **II**. Males were identified by the presence of alleles 174 and/or 176, whereas females were identified by the absence of these alleles and the presence of alleles 158 and/or 160 (Shikano et al. 2011).

To record the timing of maturation, I visually inspected each individual fish every day at two time periods. Timing of maturation was observed at two time points: at 344 DAH to 400 DAH, i.e. for a period of 8 weeks (Chapter **II**), and at 344 DAH to 512 DAH, i.e. for a period of 24 weeks (Chapter **V**). At 344 DAH to 400 DAH, 187 individuals from high feeding treatment were recorded (Hel–Hel: 44, Hel–Pyö: 48, Pyö–Hel: 49 and Pyö–Pyö: 46), and included both mature and immature individuals (Chapter **II**). At 344 DAH to 512 DAH, 282 individuals from three different feeding treatments were recorded (high feeding: 187 individuals [see above], low feeding: 35 individuals [Hel–Hel: 12, Hel–Pyö: 12, Pyö–Hel: 6 and Pyö–Pyö: 5], and recovery feeding: 60 individuals [Hel–Hel: 4, Hel–Pyö: 14, Pyö–Hel: 20 and Pyö–Pyö: 22]), and included both mature and immature individuals (Chapter **V**).

(iii) Growth

Growth was estimated using a maximum of 15 standard length measurements from photographs taken at 30, 60, 90, 120, 150, 180, 210, 240, 270, 330, 360, 390, 420, 480 and 510 DAHs. The final data set used in Chapter **IV** consisted of 192 individuals (84 males and 108 females) from the high feeding treatment. The final data set for Chapter **V** consisted of 200 individuals (136 males and 64 females) divided across the three different feeding treatments (126 individuals from high feeding, 32 from low feeding and 42 individuals from recovery feeding treatment).

(iv) Female reproductive traits

From 15 mature females of F_1 full-sib families of the marine origin (Hel–Hel; Fig. 2), the mean values of the following traits were measured: clutch size, egg size, and yolk-sac size. Mean clutch size was estimated as the mean number of eggs from the three first clutches laid by females. Both egg size and yolk-sac were measured from photographs taken using a macroscopic lens from the eggs after fertilization of the first clutch. I used the mean diameter of the eggs as a measure of egg size, and the mean diameter of the

yolk-sacs as a measure of yolk-sac size. Both, egg and yolk-sac size were measured to the closest 0.01 mm using the program TPSDIG 2 (Rohlf 2002). Clutch size was determined by counting the three first patches of eggs stripped from females. The final data set consisted of 375 records of egg measurements (25 records per female from each treatment), whilst the clutch size measurements comprised of 45 records (three records per female from each treatment; Chapter **VI)**.

(v) Early hatching larval traits

A total of 375 one day-old F_2 offspring of marine origin (Hel–Hel; Fig. 2) were collected for Chapter **VI**. The following five early hatching larval traits were measured: body size, head length, pre- and post-anal lengths, and notochord length. They were measured from photographs to the closest 0.01 mm using the program TPSDIG 2 (Rohlf 2002). The trait definitions followed those given by Jones et al. (1978; Fig. 3).

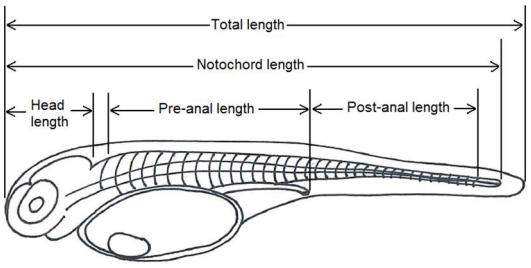


Fig. 3. Illustration of general structure and traits (*viz*. head length, pre-anal and post-anal lengths, notochord length and total length) measured from larvae used in Chapter **VI**. The measurements were made following definitions given in Jones et al. (1978).

The final data set consisted of 375 records, 25 measurements per female, from each treatment; Chapter **VI**. Since all the larval traits were highly correlated, they were collapsed into two multivariate measures of size variation using principal component analysis prior to further statistical analyses (PCA; Chapter **VI**).

(vi) Feeding behaviour

Feeding behaviour across ontogeny was quantified as the time needed for the first bite during a normal morning feeding event (Herczeg et al. 2009a; Herczeg & Välimäki 2011). Each container had a feeding hole positioned on the top, on the front side of the tank. Food was provided with a pipette through the hole, and the time that elapsed until the first biting attempt was measured with a stopwatch. If a fish did not initiate feeding after three minutes, the observation was terminated and the individuals were assigned a time of 180s. Every fish was measured at exactly the same age with a precision of one day. Feeding activity was first measured at 30 days after hatching, and the measurement was repeated monthly thereafter, resulting in nine measurements for every individual. Feeding behaviour was quantified for a total of 40 families from four cross-types (*viz*. Hel–Hel, Hel–Pyö, Pyö–Hel & Pyö–Pyö) with ten families per cross-type, and five individuals per family from the high feeding treatment. The final data set consisted of 1746 measurements divided into 194 measurements per individual from nine repeated measurements (Chapter **III**).

Statistical analyses

I used general linear models (LMs) to analyse differences in body size of females between the marine and the pond populations (Chapter **I**), and differences in body size of females between feeding treatments (Chapter **VI**). I used generalized linear mixed models (GLMMs) either with or without mean egg size as a covariate to investigate the relative influence of additive genetic, maternal, and non-additive genetic effects on body size

(Chapter **I**).

The probability of maturation (Chapters **II** & **V**) and the probability of survival (Chapter **V**) were modelled using mixed-model Cox regressions. The Cox regression model is a predictive model for right-censored time-to-event data (Cox 1972). To explore the sex differences in timing of maturation, GLMM analyses were performed for all individuals (i.e. mature and immature) and for mature individuals only (Chapter **II**). I also used GLMMs to test if there was any sex-bias in the probability of maturation, and if this differed among the cross-types (Chapter **II**).

To test for the relative influence of additive genetic, maternal, and non-additive genetic effects on individual consistency in feeding behaviour (and in the ontogeny of feeding behaviour), a repeated measures GLMM implemented in PROC MIXED (Littell et al. 2006) was used (Chapter **III**). Principal component analysis (PCA) was run to gather a reduced set of independent variables describing different aspects of the ontogeny of feeding behaviour (Chapter **III**). GLMMs on the independent PCs were used to analyse the quantitative genetic basis of feeding behaviour at different ontogenetic stages (Chapter **III**). Here, I applied Tukey–Kramer pair-wise post hoc tests to directly compare the differences in feeding behaviour among the cross-types.

To analyse individual growth trajectories (based on measurements over 15 time-points), von Bertalanffy growth curves (VB growth curves; Chapter **IV**) were fitted to the DAH. To analyse differences in the growth trajectories between populations, I used asymptotic sizes (L_∞) and intrinsic growth rates (k) as parameters (Chapter **IV**). These parameters were analysed using linear mixed effects (LME) modelling. Models were fitted separately for males and females. Next, using LME, I also investigated whether variation in individual VB growth parameters could influence maturation probability within and between populations (Chapter **IV**). Again, both sexes were modelled separately. To analyse the occurrence of compensatory growth, I analysed both size and growth measurements using both the "asynchronous" and "synchronous" approaches of Nicieza & Álvarez (2009; Chapter **V**; Box 2) using a repeated measures GLMM as implemented in PROC MIXED (Littell et al. 2006).

To investigate how feeding treatment influenced clutch size, I used GLMMs with female size and clutch number (defined as the order in which clutches were laid; from one to three) as covariates (Chapter **VI**). I also analysed variation in egg and yolk size using GLMMs (Chapter **VI**). To investigate how larval traits were influenced by feeding treatments and female traits, I analysed variation in PC1 and PC2 for larval traits in two separate GLMMs (Chapter **VI**). In order to investigate the associations between correlated female and offspring traits, I also employed structural equation modelling (SEM) as implemented in AMOS 19 (Byrne 2010).

The analyses were performed using the PASW Statistics 18 (PASW Inc. 2009; Chapters **I**, **III** & **VI**), the SAS 9.2 software package (SAS Institute Inc. 2007; Chapters **II**, **III**, **V** & **VI**), the Survival Kit v.6 software (Ducrocq et al. 2010; Chapters **II** & **V**), R 2.10.1 (R Development Core team 2009; Chapter **IV**), and AMOS 19 (Byrne 2010; Chapter **VI**).

Results and Discussion

The key specific study questions and the main findings from this thesis are summarised in Table 2. Below, I will discuss these findings and their relevance by first focussing on the relative importance of additive genetic, non-additive genetic and maternal effects as explanations for observed population divergence in different traits. After this, I will focus on what was learned about the role of direct environmental influences (i.e. phenotypic plasticity) on trait variation and population differentiation, including discussion about potential population differences in the costs of expressing phenotypic plasticity. Finally, I will discuss what was learned about the prevalence and importance of cross-generational phenotypic plasticity in a study focussed on the ancestral marine population.

The genetic basis of population differentiation

Previous research on Fennoscandian nine-spined sticklebacks using simple full-sib common garden experiments has indicated that divergence between ancestral marine and derived pond populations in a number of phenotypic traits has a genetic basis (Herczeg et al. 2009a,c; Gonda 2011; Shimada et al. 2011). Here, I found evidence for strong genetic contributions to divergence in a number of traits (body size, growth, age at maturity and feeding behaviour), but these genetic effects were not always additive. Rather, strong non-additive (dominance) contributions and maternal effects were also observed in some traits. Additive genetic effects were indicated to play a major role in body size divergence between the ancestral Baltic Sea and the derived Pyöreälampi pond population (Chapter **I**). Non-additive (dominance) effects had an influence on differences in timing of maturation (Chapter **II**), feeding behaviour across ontogeny (Chapter **III**), and intrinsic growth rate, *k* (Chapter **IV**). Maternal effects appeared to influence the differentiation in compensatory growth responses between the marine and the pond populations (Chapter **V**).

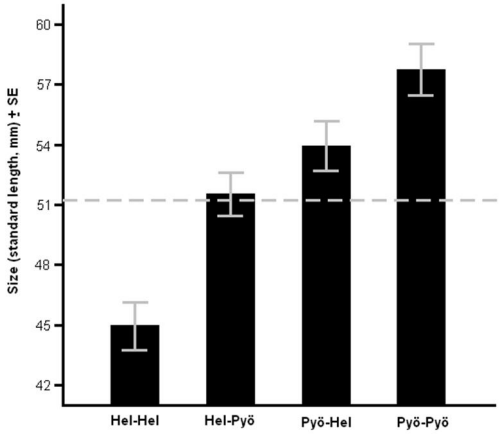


Fig. 4. Differences in the mean (± S.E.) size at 337 DAH among the 'pure' and 'hybrid' crosses of nine-spined sticklebacks. 'Hel' denotes Helsinki (the marine population) parents and 'Pyö' denotes Pyöreälampi (the pond population); the first letter denotes the father and the second the mother. The horizontal dashed line represents the expected value for the hybrids under perfect intermediacy.

In Chapter **I** I investigated body size divergence and found that gigantism in the derived Pyöreälampi pond population was based on the effects of additively acting genes, and to a minor degree, non-additive gene action (but not simple dominance) and/or maternal effects (Fig. 4). The observed minor contributions of non-additive genetic effects might also be due to the interaction between genetic and maternal effects (*cf.* Rossiter 1998), but my study design did not allow me to address this possibility further. Furthermore, given the lack of firm statistical evidence for both non-additive genetic (i.e. GLMM: marginally significant interaction

Table 2. Summary of the main study questions and results from individual Chapters (**I-VI**) included in this thesis.

	Main study questions	Main results
I	Is there a genetic basis to the body size divergence between an ancestral marine and a derived pond population of *P. pungitius*?	**Yes**. The mean body size divergence appears to be mainly due to additive genetic effects and only weakly due to maternal effects mediated through egg size.
II	Do the patterns and the genetic basis of timing of maturation differ between the marine and the pond populations?	**Yes.** The marine population matured earlier than the pond population. Early timing of maturation is a dominant trait, whereas late maturation is a recessive trait.
	Does timing of maturation differ between sexes?	**Yes.** Males matured earlier than females.
III	Is there evidence for ontogenetic shifts in feeding behaviour between marine and pond populations?	**Yes.** Feeding behaviour changed with age, and these changes were consistent across both individuals and populations.
	Are there genetically-based, ontogenetic differences in feeding behaviour between these populations?	**Yes.** The differences in feeding behaviour across ontogeny between these populations could be explained by non-additive genetic effects (i.e. genetic dominance). At the early developmental stages, the pond population expressed dominance for high feeding activity, while at the late period the marine population expressed dominance for low feeding activity.
IV	Is there a genetic basis to the differences in growth rate between marine and pond populations?	**Yes.** Analyses of von Bertalanffy (VB) growth curve parameters revealed that population differentiation in k and L_∞ has a genetic basis, but additive genetic effects do not explain all the observed differences.
	Are there sex differences in k and L_∞?	**Yes.** Analyses of VB growth curve parameters revealed that males had higher k and smaller L_∞ than females.
	Are k and L_∞ correlated, and does the correlation differ between populations?	k and L_∞ were negatively correlated within the pond, but not within the marine population.
	Does k and/or L_∞ predict age-at-maturation in *P. pungitius* populations?	**No**. Neither k nor L_∞ predicted age-at-maturation in the marine population, and only poorly so in the pond population.
V	Is there evidence for compensatory growth in *P. pungitius* populations?	**Yes.** The evidence of compensatory growth was clear in the pond population; the marine population did not exhibit compensatory growth..
	Is there a genetic basis in compensatory growth potential between the marine and the pond populations?	**Yes.** Both genetic and maternal effects mediate differences in the compensatory growth potential between populations.
	Is there a cost for compensatory growth potential, and are the costs different between populations?	**Yes.** Recovery growth delayed timing of maturation in both populations, and decreased survival in marine fish, but not in the pond population.
	Can environmentally induced plasticity obscure genetically based pattern in body size and timing of maturation between populations?	**Yes.** In both populations, individuals which were reared in a low feeding treatment were significantly smaller in body size and matured later than individuals which were reared in a high feeding treatment.
VI	Is there a cost for compensatory growth in terms of females' reproductive output?	**No**. Compensatory growth did not seem to influence female reproductive output. Recovery treatment slightly increased, rather than decreased, clutch, egg and yolk size.
	Is there a cost of compensatory growth carried into the following generation?	**Yes.** Path analytical models revealed that female size influenced offspring size independent of the effects of clutch and egg size, and these influences were found to be negative in the recovery feeding treatment.

between male origin and female origin) and maternal (i.e. analyses of intermediacy: 'hybrid' Hel–Pyö cross was close to halfway between the 'pure' Pyö–Pyö cross) effects (Chapter **I**), the most parsimonious interpretation for body size divergence between the two populations is that it is mainly a result of additive genetic effects. Hence, results give strong support for earlier conjectures postulating genetically based adaptive divergence among pond and marine populations of nine-spined sticklebacks (e.g. Herczeg et al. 2009c; Shimada et al. 2011).

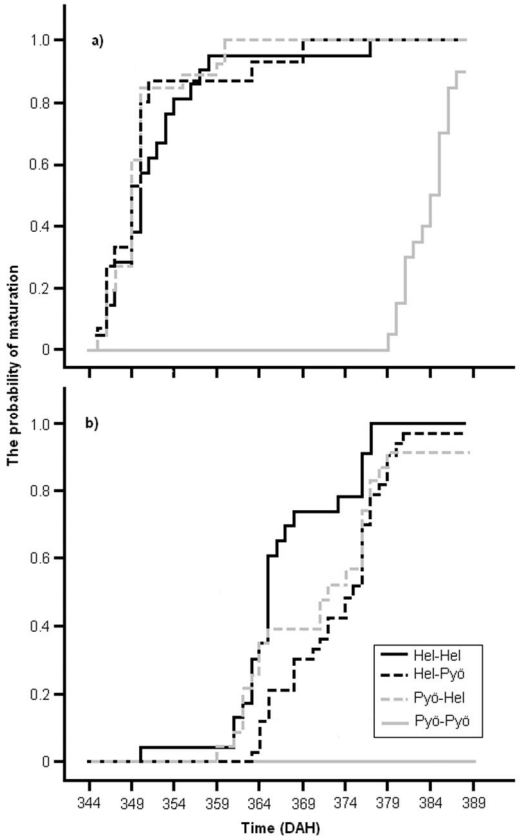


Fig. 5. The probability of maturation in the 'pure' and 'hybrid' crosses of nine-spined stickleback (**a**) males and (**b**) females as a function of time. For population abbreviations, see **Fig. 4**.

In Chapter **II** I investigated variation in timing of maturation between the focal populations. I found that early timing of maturation, a character state found in. the ancestral marine population, was a dominant trait, whereas delayed timing of maturation, a character state found in the derived pond population, was a recessive trait (Fig. 5a,b; Chapter **II**). The observed population divergence in timing of maturation suggests that strong natural selection against dominant allele(s) determining delay timing of maturation in the pond population has resulted in the accumulation of recessive allele(s) determining delayed maturation in the pond environment. The importance of dominance in determining timing of maturation has also been reported in platyfish, *Xiphophorus maculatus* (Kallman et al. 1973; Kallman & Borkoski 1978). In platyfish, five alleles at the pituitary (P) locus have been detected to be involved in determining timing of maturation: the dominant 'P1' allele determines early maturation, whilst the recessive 'P5' allele determines delayed maturation (Kallman & Borkoski 1978). In contrast, no evidence of a dominant mode of inheritance in timing of maturation was found in three populations of rainbow trout, *Oncorhynchus mykiss* (Quinton et al. 2004), and strong evidence of an additive genetic mode of inheritance in timing of maturation was found in Chinook salmon, *O. tshawytscha*, and this pattern of inheritance was hypothesized to reflect local adaptation to different spawning environments (Quinn et al. 2000).

In Chapter **III** I investigated variation in feeding behaviour, and found that differences in feeding behaviour between the focal populations were largely due to genetic dominance (Fig. 6; Chapter **III**). However, the pattern of divergence in feeding behaviour between these populations was complex, as indicated by the three-way

interaction between the origin of father, the origin of mother and the fish age. This suggests that divergence in feeding behaviour has a genetic basis, but it cannot be explained by simple additive genetic effects alone (*cf*. Lynch & Walsh 1998; Laugen et al. 2002). In addition, I also found that there was a general decrease in feeding activity with age, but these patterns differed between the two populations. Marine fish were less active than fish from the pond population during the whole observation period, but the former also showed a much stronger decrease in their feeding activity with age. This is congruent with the divergence in growth strategies observed between these populations (Shimada et al. 2011; Herczeg et al. 2012): marine fish grow quickly to a small final size, whilst the derived pond (Pyöreälampi) fish grow slowly to a large final size (Chapter **IV**). By inference, different feeding behaviours between these populations are to be expected because feeding activity has been found to be positively correlated with risk-taking and growth rate (Herczeg et al. 2009a).

Indeed, marine nine-spined sticklebacks show low feeding activity (Chapter **III**), high growth rate (Chapter **IV**) and low risk taking (Herczeg et al. 2009c), whereas the reverse is true for pond fish (Chapters **III** & **IV**, Herczeg et al. 2009c). When focussing on the ontogenetic patterns in feeding behaviour, I found that feeding activity could be separated into two main independent components: early (months 1 – 4) and late feeding activity (months 6 – 9). By comparing the hybrids to the pure crosses, an interesting pattern emerged: in both cases a non-additive genetic effect (i.e. dominance) seemed to provide the best explanation because hybrids were grouped with one of the pure crosses, while the other pure cross was clearly divergent. These comparisons suggest that at early ontogeny (months 1 – 4), high feeding activity is dominant (Fig. 7a), whilst at late ontogeny (months 6 – 9), low feeding activity is dominant (Fig. 7b).

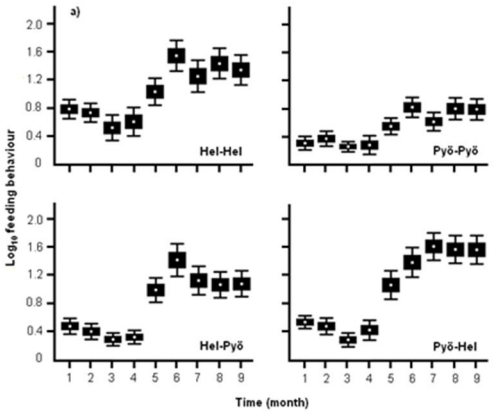


Fig. 6. Mean (± S.E. [boxes] + 95% Confidence Intervals [whiskers]) of feeding behaviour as a function of time showing ontogenetic shifts until nine months after hatching. For population abbreviations, see **Fig. 4**.

In Chapter **IV** I investigated variation in intrinsic growth rates, *k* (hereafter growth rates) between the focal populations. Divergence in growth rates between these populations has been suggested to be an adaptive response to differences in piscine predation risk, as well as to differences in the levels of inter- and intra-specific competition (e.g. Herczeg et al. 2012; Aikio et al. 2013). The results from Chapter **IV** support these adaptive explanations by showing that the growth rate differences between these populations have a genetic basis. However, it seems that the genetic factors determining growth rates in the marine population, where growth is fast, overshadow the influence of the genetic factors determining growth rates in the slow growing pond population (Fig. 8a,b,c; Chapter **IV**). In other words, as in the case of the age at maturity and feeding behaviour, the evidence from Chapter **IV** suggests a strong dominance component to growth rate divergence

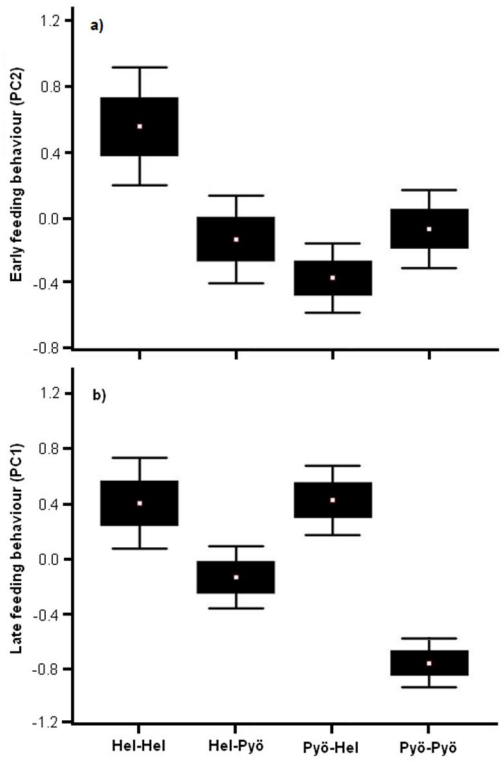


Fig. 7. Mean (± S.E. [boxes] + 95% Confidence Intervals [whiskers]) of feeding behaviour; (**a**) during early ontogeny, represented by Principal Component (PC) 2; and (**b**) during the late ontogeny, represented by PC1. Low values represent high feeding activity (short latency to feed). For population abbreviations, see **Fig. 4**.

between pond and marine populations. Furthermore, the results from Chapter **IV** are clearly incompatible with the idea that maternal effects would be important determinants of divergence in growth rate in this system.

In Chapter **V** I investigated variation in recovery growth potential between the focal populations. Recovery growth may be an adaptive mechanism that allows organisms to endure early life starvation and catch-up in size later on (Metcalfe & Monaghan 2001). It is commonly observed in animals such as fish in conjunction with early-life nutritional restrictions (e.g. Ali et al. 2003). There are two possible mechanisms behind recovery growth: catch-up growth and compensatory

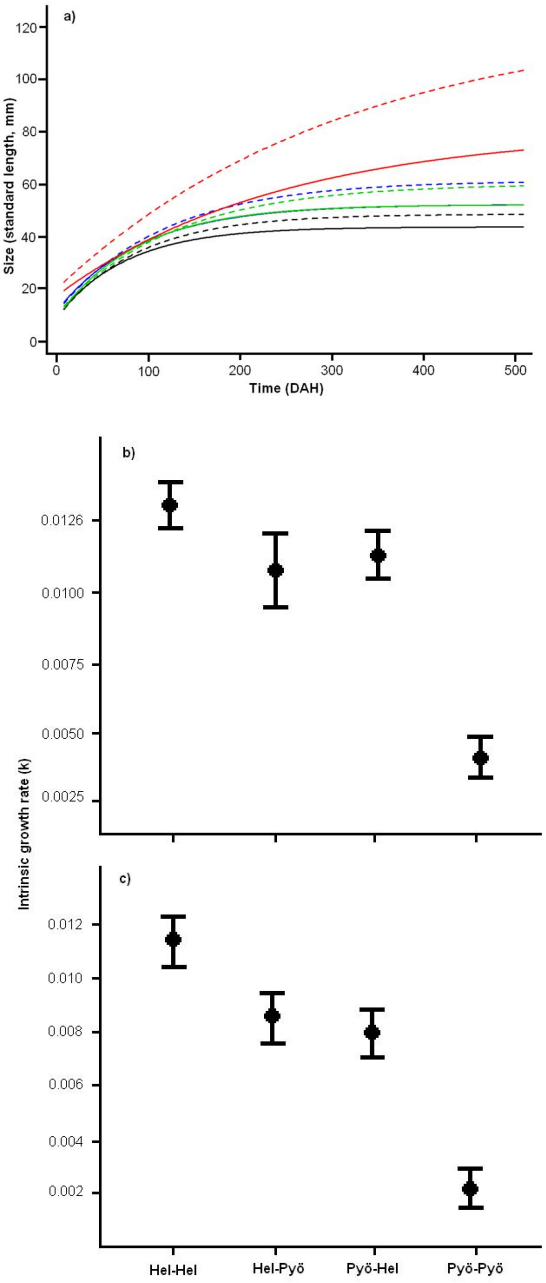


Fig. 8. (**a**) Mean von Bertalanffy growth trajectories of four cross-types of nine-spined sticklebacks. Trajectories were fitted through non-linear least-squares regression separately for Hel-Hel males (smooth black line), Hel-Hel females (dashed black line), Hel-Pyö males (smooth blue line), Hel-Pyö females (dashed blue line), Pyö-Hel males (smooth green line), Pyö-Hel females (dashed green line), Pyö-Pyö males (smooth red line), and Pyö-Pyö females (dashed red line). Mean (± 95% CI) intrinsic growth rates (log-transformed *k*) for (**b**) males, and (**c**) females. For population abbreviations, see **Fig. 4**.

growth (Jobling 2010; Box 2). In Chapter **V** I found evidence for the existence of both compensatory and catch-up growth in nine-spined sticklebacks, and that maternal effects might possibly influence compensatory growth divergence between the marine and pond populations (Chapter **V**). Interestingly, fish from the marine population did not exhibit compensatory growth (i.e. growth rate acceleration over routine growth rates; Ali et al. 2003), but showed over compensation: individuals from the recovery treatment grew larger than those from the high feeding treatment (Fig. 9a,b). In contrast, fish from the pond population showed full (but not over) compensation: individuals from the recovery treatment grew to become as large as those from the high feeding treatment (Fig. 9a,b). It is worth noting that examples of over compensation are extremely rare, and even full compensation is seldom observed/reported (reviewed in Ali et al. 2003).

Phenotypic plasticity

Previous studies of Fennoscandian nine-spined sticklebacks indicate that biotic factors such as predation and competition have significantly promoted divergence between ancestral and derived populations in a number of traits (reviewed in Välimäki 2012; Merilä 2013). This implies that apart from genetically-based local adaptation, direct environmental effects may also have influenced the divergence between these populations.

In the last two chapters of this thesis (**V** and **VI**), I explored phenotypic plasticity in growth, and the potential costs of this plasticity. In Chapter **V** I found that fish from the pond population accelerated their growth above their routine levels when exposed

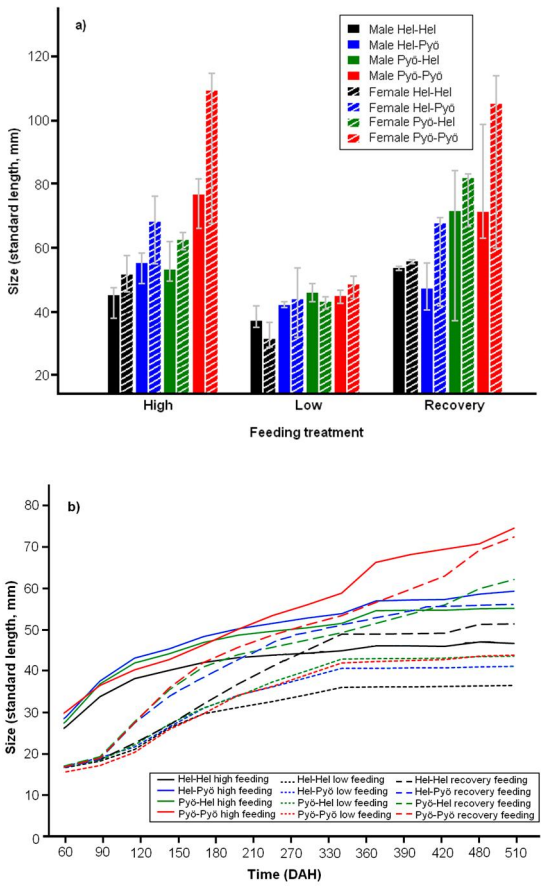


Fig. 9. (**a**) Mean (± SE) body size among four cross-types of nine-spined stickleback in different feeding treatments. The histograms were fitted through the last observation of mean body size measurement at 510 DAH in high, low and recovery feeding treatments separately for each cross-type. (**b**) Growth increments in four cross-types of nine-spined sticklebacks in different feeding treatments. Trajectories were fitted through actual mean body size measurement in high, low and recovery feeding treatments separately for each cross-type. For population abbreviations, see **Fig. 4**.

to recovery feeding treatment, while fish from the marine population did not (Fig. 10). This result contradicts previous findings showing that populations exhibiting high, rather than low, growth rates often show evidence for compensatory growth (Schultz et al. 2002; Fraser et al. 2007). However, this contradiction between these and the earlier results may be more apparent than true for a number of reasons. For instance, in the case of fast growing

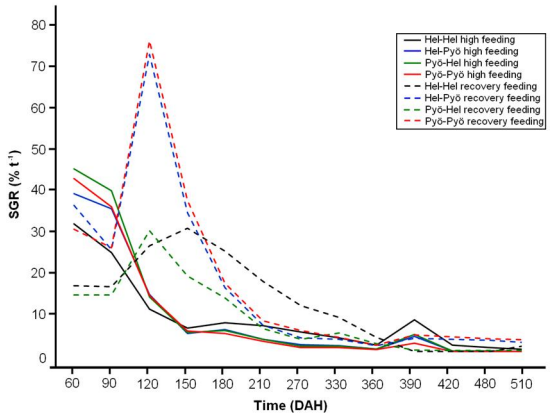


Fig. 10. Specific growth rates (*SGR*) at different time intervals calculated from the data shown in Fig. 9b. For population abbreviations, see **Fig. 4**.

marine nine-spined sticklebacks, high predation pressure from piscine predators is likely to favour early maturation at a small size (Herczeg et al. 2012; Aikio et al. 2013), and select against strong compensatory growth responses. This is because fast growth requires increased activity and movements which in turn increase the risk of mortality through predation (Biro & Post 2008; Biro & Stamps 2008). Hence, the absence of a compensatory growth response in marine fish could reflect adaptive restraint towards a response which might result in increased mortality risk. Furthermore, it is a fact that selection has favoured larger body size in the pond populations and smaller body size in the marine population (e.g. Herczeg et al. 2010a, 2012; Karhunen et al. 2013; Merilä 2013). Hence, given the importance of reaching large size in the pond habitat, one might expect selection also to favour evolution of compensatory growth responses in the ponds as such responses would allow fish to capitalize on periods of food abundance to maximize their size at maturity. Whatever the ultimate cause for the observed differences, the fact remains that the pond nine-spined sticklebacks are apparently able to accelerate their growth in response improved feeding conditions following a period of food limitation. Moreover, this ontogenetic plasticity in growth rates may be adaptive.

In Chapter **V** I also found that the degree of plasticity in growth patterns, and the costs of expressing them, seemed differ between marine and pond populations. In the early maturing marine population, lack of a compensatory growth response was observed to be associated with the occurrence of over compensation – individuals subject to recovery food treatment did not exhibit growth acceleration, but eventually grew to a larger size than those maintained in the high feeding treatment (Fig. 9a,b). This over compensation in the marine population was associated with reduced survival (Fig. 11a) and delayed maturation probability (Fig. 11b,c; Chapter **V**) among the fish from the recovery feeding treatment. In contrast, a clear compensatory growth response occurred in the late maturing pond population which in turn led to full size compensation at the end of the experimental period (Fig. 9a,b). The cost of this plasticity appeared to be restricted to delayed maturation (Fig. 11b,c; Chapter **VI**). Hence, in both populations, the recovery feeding treatment resulted in a significant reduction in maturation probability, suggesting a cost to early life feeding restriction. Moreover, the recovery feeding treatment also reduced survival probability in the marine population, but not in the pond population. In addition to demonstrating evidence for possible adaptive plastic responses to early life food restriction, these findings also lend support for earlier observations demonstrating maturation and survival costs for recovery growth

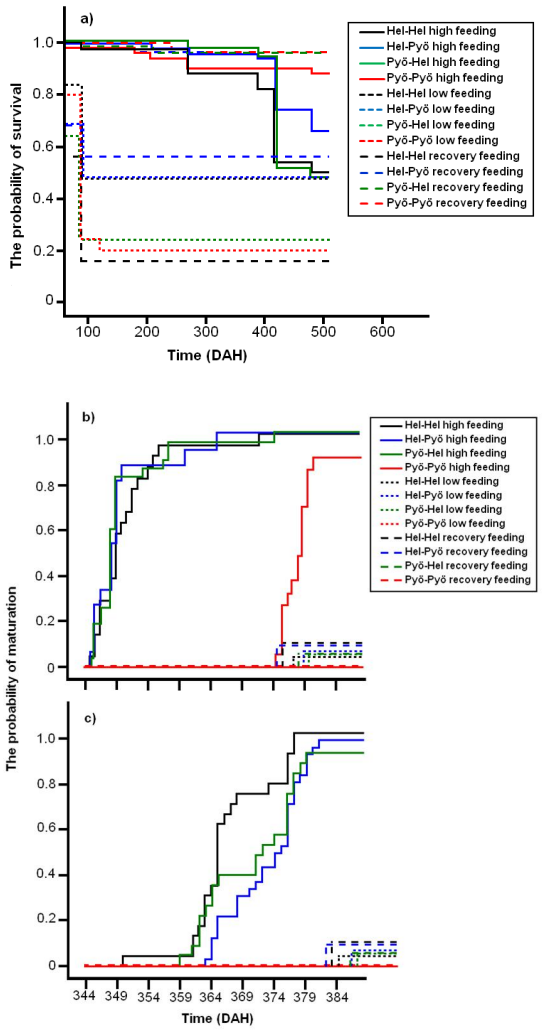


Fig. 11. (**a**) The probability of survival among four cross-types of nine-spined sticklebacks after recovery growth. Trajectories were fitted separately for each cross-type. The probability of maturation among four cross-types of nine-spined sticklebacks (**b**) males, and (**c**) females. Trajectories were fitted separately for each cross-type. For population abbreviations, see **Fig. 4**.

responses (Metcalfe & Monaghan 2001; Ali et al. 2003).

Cross-generational phenotypic plasticity

Phenotypic plasticity can be restricted to one generation, or a certain developmental period, but it can also persist over several generations via cross-generational plasticity (Rossiter 1996; Mousseau & Fox 1998; West-Eberhard 2003, p. 141; Salinas & Munch 2012). It is well known that parental (usually maternal) environment can substantially affect offspring traits (e.g. Roach & Wulff 1987; Rossiter 1996; Mousseau & Fox 1998; Salinas & Munch 2012). By extension, maternal effects can be viewed as a form of cross-generational plasticity in which the phenotype of the offspring is influenced by the environmental conditions experienced by their mothers (Rossiter 1996). In Chapter **VI** I explored the occurrence of cross-generational plasticity in nine-spined sticklebacks by focussing on the effects of feeding treatments on female size, female reproductive traits (*viz.* clutch size, egg size and yolk size), and how these traits influence offspring size.

I found that the feeding treatments had effects on all female reproductive traits, mainly due to the negative effects of low feeding treatment on female size, which in turn had a negative effect on clutch size (Chapter **VI**). However, after controlling for influences of female size on clutch size, it turned out that the females subjected to the recovery feeding treatment produced larger clutches than those subjected to high and low feeding treatments (Fig. 12). This finding does not give support for the expectation that compensatory growth would have incurred costs in terms of reduced fecundity (Chapter **VI**) – a finding in agreement with some earlier results (Dmitriew & Rowe 2007; but see: Auer et al. 2010).

However, the results of the structural equation modelling revealed that the size of the offspring of females from the recovery feeding treatment was negatively impacted by the size of the females, whereas this effect was positive in the high and low feeding treatments (Fig. 13a,b,c). This suggests a cross-generational cost of

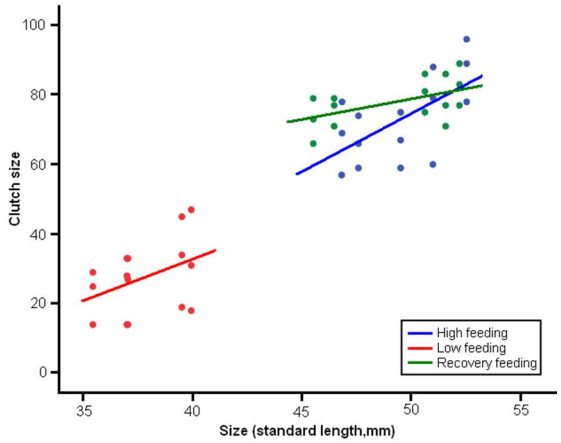


Fig. 12. Clutch size as a function of female size in nine-spined sticklebacks in high, low and recovery feeding treatments, respectively. Plotted are the values for three replicate clutches for five females from the three different treatments. Data from Chapter **VI**

compensatory growth. This interpretation is supported by the fact that this effect is independent of the direct effects of clutch, egg and yolk size on offspring size (Fig. 13a,b,c).

Conclusion and Future Directions

In conclusion, using laboratory experiments and controlled crosses, I have demonstrated that population divergence in several ecologically important phenotypic traits have a genetic basis in the nine-spined stickleback. Furthermore, I have shown that while the mode of inheritance underlying population differentiation in some of the traits (e.g. body size) appears to be mostly additive, in other traits (e.g. timing of maturation, intrinsic growth rates and feeding behaviour) non-additive genetic contributions seem to be important. Likewise, in the case of some of the traits, maternal effects also contribute – albeit only weakly – to observed population differentiation. I also found that environmental influences have potential to cause population divergence in this species: by manipulating feeding

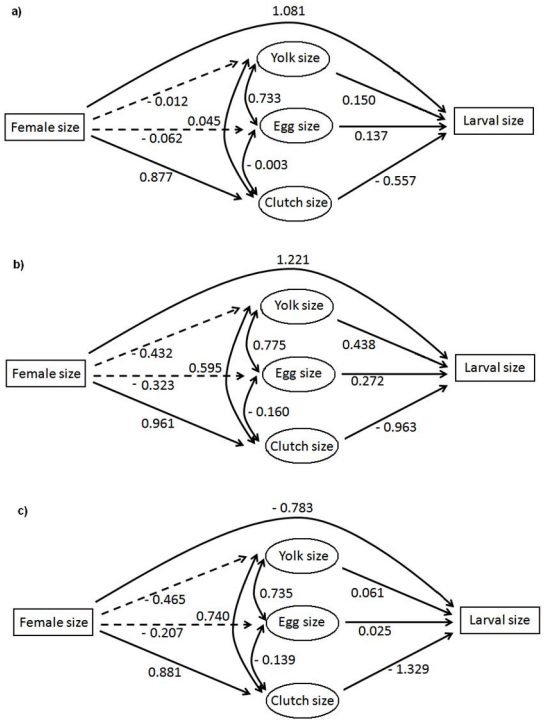


Fig. 13. Results of structural equation model (SEM) showing the effects of female size, yolk size, clutch size and egg size on larval size of nine-spined sticklebacks in (**a**) high, (**b**) low, and (**c**) recovery feeding treatments. Single-headed arrows indicate significant paths: significant paths are displayed as intact lines, whilst non-significant paths are displayed as dashed lines. The double-headed arrow indicates correlations between variables.

regimes, I found that growth rates and body size were highly plastic, and environmental influences induced by feeding treatments can persist through generations. In general, the results support the earlier inference – based on rearing F_1, intra-population, full-sib crosses (e.g. Herczeg et al. 2009c; Shimada et al. 2011; Herczeg et al. 2012) – that much of the phenotypic differentiation seen in the wild (and in the earlier common garden studies) indeed has a genetic basis. As such, the results add weight to interpretations according to which the observed divergences reflect local adaptation to habitat differences in piscine predation pressures, as well as to differences in

the degree of inter- and intra-specific competition among habitats (e.g. Herczeg et al. 2009c; Karhunen et al. 2013). In addition, the results of the two last chapters provide evidence for the possible adaptive – and hence also genetically based – population differentiation in compensatory growth potential. They also identify and demonstrate costs to compensatory growth responses. Given that similar studies in an inter-population context are still rare, they should provide a valuable addition to our understanding of the costs and evolution of compensatory growth responses. With respect to the specific case of isolated pond populations of the nine-spined stickleback, these results add further weight to the argument that these populations are so genetically and phenotypically distinct from their putative ancestral source that they should perhaps receive special attention in a conservation context (*cf*. Merilä 2013).

There are a couple of clear avenues to further refine our understanding of the nature and genetic underpinnings of the phenotypic divergence among pond and marine nine-spined sticklebacks. One is to produce F_2 backcrosses between F_1-hybrids (e.g. Pyö–Hel) and pure parental (e.g. Pyö–Pyö) crosses. This was indeed my intention at the beginning of my thesis work, but various logistical constraints (e.g. problems with obtaining mature F_1 pond females; **II**) lead me to abandon this line of research. However, with careful planning, these back-crosses could be obtained allowing more refined dissection of non-additive and maternal effects from each other (e.g. Jinks 1956; de Belle & Sokoiowski1987; Carroll et al. 2001; Huttunen & Aspi 2003). Naturally, albeit logistically challenging, use of replicate marine and pond populations would help to generalize these findings with respect to habitat type effects, and also, to explore the possible within habitat heterogeneity in genetic and plastic underpinnings of trait variability.

Another obvious line of future research resides in the possibilities afforded by genomic approaches to identifying causative (or linked) loci underlying observed phenotypic divergence (e.g. Fournier-Level et al. 2011; Fan et al. 2012). As a matter of fact, several QTL-studies of nine-spined sticklebacks have surfaced recently (e.g. Shapiro et al. 2009; Shikano et al. 2013; Laine et al. 2013a,b). However, denser linkage maps and other types of mapping approaches (e.g. genome-wide association studies, GWAS; e.g. Johnston et al. 2011) are required before the genetic underpinnings of complex polygenic traits such as body size are likely to be deciphered. Until then, classical quantitative genetic approaches such as those used in this thesis, in combination with the application of evolutionary null-models (e.g. Karhunen et al. 2013), can still provide useful insights into the causes and adaptive nature of phenotypic population differentiation.

Acknowledgements

I would like to express my sincere appreciation and gratitude to my main supervisor, Professor Juha Merilä and co-supervisor, Dr. Tuomas Leinonen for providing me with the opportunity to conduct my PhD research project in the University of Helsinki and for providing support, advice and encouragement at all stages of my PhD study. I was a – wanna be – scientist without much experience and my English was poor. Their insightful suggestions and instructive comments contributed

greatly to the evolution of my research and development as a scientist. Thank you for believing in me and in our research projects.

I would like to thank my graduate thesis committee members Dr. Heikki Hirvonen and Dr. Ulrika Candolin for their helpful suggestions throughout my time as a PhD student.

I would like to thank Dr. Gábor Herczeg for his support, guidance and collaboration throughout the years. Dr. Gábor Herczeg had supervised me at the beginning of my PhD study. Thank you for introducing me to fieldwork in Finland, common garden practice, geometric morphometric and behavioural study. Your constant support, encouragement, availability and humanity mean a lot to me.

I would like to express my sincere appreciation and gratitude to my colleague from EGRU of past and present (Dr. Abhilash Nair, Dr. Abigél gonada, Bineet Panda, Dr. Cécile Patrelle, Dr. Graciela Sotelo, Heini Natri, Dr. Jussi Alho, Dr. Kaisa Välimäki, Kristina Noreikiene, Dr. Meng-Hua Li, Niina Nurmi, Dr. Yukinori Shimada), and dear Malaysian friends here in Helsinki and abroad for their support during the four years of my PhD for all their patience, for listening to my moaning and complaining and supporting me every step of the way and teaching me Finland. Thank you for helping to keep me sane throughout the years and for bringing new adventures to my life.

Thank you to the native English speakers: Dr. John Loehr, Dr. Jacquelin DeFaveri and Dr. Scott McCairns for correcting my English – usually at a very short notice (I have still a long way to go to write and speak properly English).

I would like to thank all the EGRU supportive staffs that were always ready to help even at the last-minute. Thank you to EGRU secretaries, Linda Uoti, Dr. Maarit Haataja and Marika Hölttä. Thank you to EGRU laboratory technicians, the present: Kirsi Kähkönen and the past: Marika Karjalainen. Thank you also to EGRU part-timer and master student, Mirva Turtiainen.

This PhD would not have been possible without the tremendous financial support from the Academy of Finland (grants #129662, #134728, #218343, # 250435 and #265211 to JM and #128716 to GH), as well as University Putra Malaysia and LUOVA graduate school to me. The research leading to these results has also received funding from the European Community's Seventh Framework Programme (FP/2007–2013) under grant agreement n°217246 through BONUS, the joint Baltic Sea research and development programme (to JM).

Finally, I would like to express my gratitude to my parents for providing unwavering support in all of my life decisions, and especially when they learned I was going abroad and may not return to Malaysia in the near future! I also would like to thank my best friends – my brothers for their incredible support and encouragement.

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“This manuscript provides an interesting contribution on the evolution of phenotypic divergence in recent, i.e. post-glacial systems. The experimental approach and the statistical analyses are sound...”
- *Biological Journal of the Linnean Society*

“This study was interesting and its emphasis on maternal effects a good addition to the rather tiresome common garden approach of separating environmental versus genetic variation in a trait”
- *Biological Journal of the Linnean Society*

“This is a solid study that investigates the genetic basis of life-history variation, and sex-specificity of such variation...”
- *Journal of Evolutionary Biology*

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ISBN 978-952-10-9690-7 (paperback)

ISBN 978-952-10-9691-4 (PDF)

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