# Evolutionary Genetics in the Wild – from Populations to Individuals

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Academic dissertation

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## Contents

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IV Leinonen, T., Cano, J.M. & Merilä, J. Genetic basis of sexual dimorphism in the threespine stickleback. *Manuscript.*

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## Contributions

| | I | II | III | IV | V | VI |
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| Concept | TL, JC, JM | JM | TL, JC, JM | TL | GH, JL, JM | TL, JC |
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# Abstract

Predicting evolutionary outcomes and reconstructing past evolutionary transitions are among the main goals of evolutionary biology. Ultimately, understanding the mechanisms of evolutionary change will also provide answers to the timely question of whether and how organisms will adapt to changing environmental conditions.

In this thesis, I have investigated the relative roles of natural selection, random genetic drift and genetic correlations in the evolution of complex traits at different levels of organisation – from populations to individuals. I have shown that natural selection has been the driving force behind body shape divergence of marine and freshwater threespine stickleback (*Gasterosteus aculeatus*) populations, while genetic drift may have played a significant role in the more fine scale divergence among isolated freshwater populations. These results are concurrent with the patterns that have emerged in the published studies comparing the relative importance of natural selection and genetic drift as explanations for population divergence in different traits and taxa.

I have also shown that body shape and armour divergence among threespine stickleback populations is likely to be biased by the patterns of genetic variation and covariation. Body shape and armour variation along the most likely direction of evolution – the direction of maximum genetic variance – reflects the general patterns of variation observed wild populations across the distribution range of the threespine stickleback. Conversely, it appears that genetic correlations between the sexes have not imposed significant constraints on the evolution of sexual dimorphism in threespine stickleback body shape and armour.

I have demonstrated that the patterns of evolution seen in the wild can be experimentally recreated to tease out the effects of different selection agents in detail. In addition, I have shown how important it is to take into account the correlative nature of traits, when making interpretations about the effects of natural selection on individual traits. Overall, this thesis provides a demonstration of how considering the relative roles of different mechanism of evolutionary change – at different levels of organisation – can aid in an emergence of a comprehensive picture of how adaptive divergence in wild populations occurs.

# Introduction

Understanding how organisms adapt to changing environments is an integral part of biology, extending from basic ecology and conservation biology to agriculture and medicine. Adaptation to changing environments entails evolutionary change, which is dependent on heritable variation. Evolutionary genetics provides tools for quantifying variation at different levels of resolution, from populations to individual loci, and serves as a platform from which to reach for one of the most important goals of evolutionary biology – predicting and reconstructing evolutionary change in wild populations.

In its very essence, evolution can be defined as a change in phenotypic means, which is a function of genetic drift, natural selection and the matrix of genetic variances and covariances, G (Polly 2008). These three components of evolution are intertwined so that population size and G determine genetic drift, while an adaptive landscape in terms of fitness determines the strength and direction of selection (Lande 1976, Arnold et al. 2001, Gavrilets 2004). Predicting evolutionary change in wild populations includes estimating all the above three essentials, which in turn requires investigation of variation at different levels of resolution.

## *Variation between populations - drift or selection?*

When starting to uncover the details of variation observed in nature, the foremost issue to consider is that evolution usually takes place on such a long time scale that the observed patterns of phenotypic change could be explained by very little selection or even by genetic drift alone (Lande 1976, Lynch 1988, Kinnison & Hendry 2001; but see Estes & Arnold 2007). Genetic drift is more likely to play a larger role in populations with small effective population sizes ($N_e$), where the rate of genetic drift is higher than in populations with large $N_e$. The higher rate of genetic drift in small populations provides ample scope for non-adaptive differentiation, which in turn decreases the efficiency of natural selection. It can thus be said that the efficiency of natural selection is inversely related to $N_e$ (e.g. Jones et al. 1968, Frankham et al. 2002, England et al. 2003).

The relative importance of natural selection and genetic drift in explaining evolutionary change has constituted a long-standing debate in evolutionary biology (e.g. Merilä & Crnokrak 2001, McKay & Latta 2002). The debate has remained unresolved, although statistical tools for testing the differentiation have been around for some time (Lande 1976, 1977, Chackraborty & Nei 1982, Lynch & Hill 1986, Lynch 1988, Turelli 1988). The problem with these tools is their inherent assumptions on critical parameters, such as mutational input, time since population divergence and population size, which are often impossible to quantify (e.g. Turelli 1988, Lande 1992, Lynch 1994). However, there is an alternative approach, which is based on comparison of genetic differentiation in neutral marker loci ($F_{ST}$) and quantitative traits ($Q_{ST}$; for details on the calculation of the indices see Box 1). This method has also been available for as long as the first mentioned method (Wright 1951, 1965, Rogers & Harpending 1983, Felsenstein 1986), but for some reason – perhaps partly due to early criticism by Lewontin (1984) on the actual information content of the marker vs. quantitative trait comparisons – it has not been widely adopted prior to the recent decade or so. Another factor that has possibly hindered the usage of the $F_{ST}$ vs. $Q_{ST}$ comparisons, is that estimating the index of quantitative genetic differentiation requires teasing out the genetic component of variance, which requires controlled breeding experiments or known pedigrees of large sample size and a large number of populations (Lynch & Walsh 1998, O'Hara & Merilä 2005).

$F_{ST}$ and $Q_{ST}$ estimates are based on the relationship of between-population and within-population variance, so that the estimate of population differentiation in a quantitative trait, $Q_{ST}$, is analogous to population differentiation at a single locus $F_{ST}$ (Spitze 1993). There are three possible outcomes of the $F_{ST}$ vs. $Q_{ST}$ comparisons with different evolutionary implications (Merilä & Crnokrak 2001). First, if $Q_{ST}$ is higher than $F_{ST}$, directional natural selection must have played a role in the observed population divergence. In this case selection is not favouring similar phenotypes in different populations, which has been the most common outcome in empirical studies (Merilä & Crnokrak 2001). The second possible outcome is that the indices of divergence are equal ($F_{ST} \approx Q_{ST}$). This can be interpreted so that the observed degree of differentiation could have been a product of genetic drift alone. In other words, selection may have been in action, but its effects cannot be distinguished from drift. The third possible outcome, $F_{ST} > Q_{ST}$ means that the degree of differentiation is less than what would be expected by genetic drift alone. The interpretation of this situation is that natural selection favours similar phenotypes in different populations. In a rare case, this outcome could reflect a situation, where there is not enough genetic variability in the studied populations so that natural selection does not have any variance to act on (Merilä & Crnokrak 2001).

It must be noted that the approach of comparing the quantitative genetic and neutral genetic differentiation has a number of inherent limitations in both the estimation of $F_{ST}$ (e.g. Hedrick 1999, Meirmans 2006) and $Q_{ST}$ (e.g. O'Hara & Merilä 2005, Pujol et al. 2008, Whitlock 2008). Despite these possible problems, the method of comparing $F_{ST}$ vs. $Q_{ST}$ estimates provides a good platform for exploring the forces that are in play in phenotypic divergence, and thus, is a natural starting point for the investigation of phenotypic divergence in more detail.

### *Variation within populations - genetic variance and covariance structure*

Whether the driving force for phenotypic change is natural selection or genetic drift, there will be no evolutionary change if there is no heritable genetic variation. In the simplest case, the interest is on a single trait and evolution (change in the mean of a trait – response, $R$) can be summarised by the breeder's equation:

$$R = h^2 S$$

where $h^2$ denotes the heritability of the trait and $S$ the selection differential. As the name implies, the origins of the equation lie in animal breeding, where the interest is usually in improving a single trait. However, predicting evolution in wild populations usually involves considering a number of traits simultaneously. In addition, selection rarely, if ever, acts on a single trait alone (Lande 1986), and evolutionary change in a heritable trait is not free to take a direct pathway to its optimal phenotype as imposed by natural selection. The paths of evolution are bounded by the constraints set by the patterns of genetic variation and development (Bonner 1982, Maynard Smith et al. 1985), which is the reason why it is necessary to have information on genetic variances and covariances of the traits when trying to unravel the details behind phenotypic variation.

The influence of genetic covariance between traits on evolution can be summarised with the multivariate extension of the breeder's equation (Lande 1979, Lande & Arnold 1983):

$$\Delta z = GP^{-1}s$$

which returns a vector of changes in mean trait values ($\Delta z$), and where G is the matrix of genetic variances and covariances, P its phenotypic equivalent and s is the vector of selection differentials. To spell it out, G describes linkage or pleiotropic connections between traits that might cause correlated patterns of evolution in the traits. P depicts phenotypic relations among traits that result from the interplay of the genetic covariance matrix and the developmental processes influenced by the environment.

Estimation of the heritable component of variation requires either controlled breeding experiments in the lab, sampling in the wild so that the pedigree matches a standard design (such as parent-offspring), or using an animal model for a population sampled over multiple generations (reviewed by Roff 2007). Unfortunately, none of the above-mentioned methods are free of problems. Common, and perhaps the most critical problem with all quantitative genetic parameter estimation methods is that they require a rather large sample size (Lynch & Walsh 1998). With a single or a limited number of traits, it is possible to get estimates with reasonable power, but as the number of traits increases, so does the required sample size, approximately as the quartic root of the number of traits (Phillips 1998). In addition, the power to detect significant heritability decreases when the heritability estimates get smaller (Lynch & Walsh 1998), a situation to be expected when the complexity of traits increases, for example as in life history traits (Price & Schluter 1991, Merilä & Sheldon 1999). As a result, studies of genetic correlations have traditionally been restricted to consider a very limited number of traits. However, the integration of quantitative genetics with geometric morphometric methods (Box 2), has paved the way for quantitative genetic investigation of complex (and comprehensive) traits, such as body shape (Klingenberg & Leamy 2001, Monteiro et al. 2002, Klingenberg & Monteiro 2005, McGuigan et al. 2005).

It must be noted though that single trait cases are not free of problems either. Heritability estimates can easily be misinterpreted, if one does not keep in mind that they are specific to population, environment, generation, age and model used for their estimation (e.g. Hoffmann & Merilä 1999, Charmantier & Garant 2005, Wilson 2008). The issues that undermine the usefulness of heritability in predicting evolution are also reflected in the analysis of multiple traits, which possibly complicates the use of G in reconstructing the patterns of evolution that have led to the phenotypic differentiation observed today (e.g. Pigliucci 2006). The usefulness of G depends on its stability over time (Turelli 1988) – an issue as yet unresolved (Roff 2000, Steppan et al. 2002, but see Björklund 2004). One solution to this possible problem is to use the G of an ancestral population, which unfortunately is rarely possible. Of course, the stability of G over time and in different populations depends on the traits in question. For instance, if the genetic covariances result from a pleiotropy among numerous alleles at

Box 1. Glossary – abbreviations of quantitative genetic concepts

| | |
|---|---|
| $N_e$ | Effective population size – the average size of a population in terms of individuals that can contribute genes equally to the next generation |
| FA | Fluctuating asymmetry – small, non directional departures from perfect symmetry |
| $F_{ST}$ | Measure of genetic differentiation in neutral marker genes[1,2]<br>$F_{ST} = \frac{V_b}{(V_b + V_w)}$<br>$V_b$ = variation between populations<br>$V_w$ = variation within populations |
| $g_{max}$ | Direction of maximum additive genetic variance or 'genetic line of least resistance'. The dominant eigenvector of the G matrix[3] |
| G | Matrix of genetic variances and covariances, calculated based on phenotypic similarity among relatives.<br>$G = \begin{vmatrix} \sigma^2_{A(1)} & \sigma_{A(1,2)} & \dots & \sigma_{A(1,j)} \\ \sigma_{A(2,1)} & \sigma^2_{A(2)} & \dots & \sigma_{A(2,j)} \\ \dots & \dots & \dots & \dots \\ \sigma_{A(i,\,1)} & \sigma_{A(I,2)} & \dots & \sigma^2_{A(n)} \end{vmatrix}$<br>$\sigma^2_{A(i)}$ = variance of trait i<br>$\sigma_{A(i,j)}$ = covariance between traits i and j |
| $h^2$ | Heritability – proportion of phenotypic variation in a population that is attributable to genetic variation among individuals<br>$h^2 = \frac{V_A}{V_P}$<br>$V_A$ = additive genetic variation, $V_P$ = phenotypic variation |
| P | Matrix of phenotypic variances and covariances (see G for an example) |
| $Q_{ST}$ | Measure of differentiation in quantitative traits[4]<br>$Q_{ST} = \frac{V_b}{(2V_b + V_w)}$<br>$V_b$ = variation between populations<br>$V_w$ = variation within populations |
| $S$ | Selection differential – difference between phenotype of selected parents and population mean |
| s | Vector of selection differentials |

References: 1. Wright (1951), 2. Nei (1987), 3. Schluter 1996, 4. Spitze (1993)

### Box 2. A primer to geometric morphometrics

Geometric morphometrics uses outline or landmark based methods (Adams *et al.* 2004). Landmark analysis relies on biologically definable points, such as points of intersection of bones, which are homologous across all specimens. This makes biological interpretations more holistic, which is the main advantage of landmark-based methods (Rohlf & Marcus 1993). Reyment (2002) defines landmarks as recognizable equivalent points observed on the objects being compared. For each specimen, the landmarks are given coordinates (x and y) on a coordinate plane (coordinates can also be in a three-dimensional plane, when the z coordinate is included). When all the specimens have been given corresponding landmarks, differences in coordinates for each landmark can be compared. This analysis begins with superimposing the landmark configurations of each specimen on each other. Superimposition basically means overlaying one specimen's coordinates on top of another's, so that their homologous landmarks match as closely as possible (Rohlf & Marcus 1993). The aim of superimposition is to remove non-shape variation from a set of data. Non-shape variation is due to changes in translation, rotation, and scale of specimens (Slice *et al*. 1996). The most common of the superimposition methods used today are based on least-squares methods (also called Procrustes analysis; Slice 2001, Adams *et al.* 2004), which estimate the parameters for location and orientation that minimise the sum of squared distances between corresponding points on two configurations (Bookstein 1991). This is done by first translating the centroid of each configuration to the origin and scaling the configurations according to their centroid sizes (Rohlf & Slice 1990). Centroid is essentially the centre of gravity of a specimen as derived from its landmark configuration, and centroid size is the square root of the sums of squared distances of a set of landmarks from their centroid (Slice *et al*. 1996). Superimposition is completed by rotating the configurations so that the squared differences between corresponding landmarks are minimized (Rohlf & Slice 1990). With every specimen the process is iterated to get the mean (reference) shape, which cannot be estimated before superimposition (Adams *et al*. 2004).

After superimposition, the landmark configurations lie on a common coordinate system and the coordinates of landmarks can be used as shape variables (Adams *et al*. 2004). The whole range of multivariate statistics can be used to explore the structure of shape variation. Once the statistical analysis is done, and here lies the beauty of geometric morphometrics, the results can be easily visualised in the space of the original specimens. The most widely used visualisation method today is thin-plate spline (Reyment 2002). Landmarks on a thin-plate spline are thought to lie on an indefinitely thin metal plate. For example, if we had two specimens, one's landmark configuration would lie on a straight plate, while the plate would be bent to fit the configuration of the other one. This new surface should be the least bent of any surface that passes through the specified heights at the locations of the reference landmarks (Bookstein 1991). The difference in the shape of the two specimens can then be expressed as the energy needed to bend the straight plate to its new shape. This is easiest to visualise if a grid is used as a plate. Bending of the grid shows where the variation in specimens lies.

The results of thin-plate spline analyses are usually expressed as warps. Thin-plate spline analysis takes into account the variation at each landmark separately. At each landmark, the difference of a specimen from the mean configuration is called a partial warp. Partial warps are vectors that lie on a coordinate plane defined by principal warps, which in turn are orthogonal shape axes that represent all possible deformations that could occur starting from the reference configuration. When partial warps are used in a principal component analysis together, so that total shape variation is taken into account, the resulting differences are called relative warps. Scores from the relative warp analysis can be plotted on a grid, where deformation of the grid reflects the variation in the shape of the specimens.

When morphological data are compared with genetic data, it is useful to have an overall measure of shape variance. Analyses based on Procrustes distance have been previously used to assess the magnitude of individual variation, fluctuating asymmetry and measurement error (Klingenberg & McIntyre 1998). Procrustes distance has been also used in quantitative genetics (Monteiro *et al.* 2002), although with some criticism (Klingenberg 2003). Procrustes distance is the square root of the sums of squared differences between corresponding landmarks (Rohlf 1999). It therefore combines all the variation in the landmarks to produce a single measure of overall shape variability between specimens, which can then be compared for example with indices of genetic variability, such as $F_{ST}$ (see Chapter I).

each locus with a multinormal distribution of effects, the G matrix is likely to have remained relatively constant, even under changing selective pressures (Blows & Higgie 2003).

When the G matrix is assumed to be constant, genetic covariances are likely to constrain evolution so that phenotypic change is most likely to take place along the direction of maximum additive genetic variance ($g_{max}$), which in essence is the dominant eigenvector of the G matrix (Schluter 1996). However, apart from Schluter's (1996) original tests on evolution along the lines of least resistance, very few studies have found a positive association between the $g_{max}$ and phenotypic divergence (but see Arnold & Phillips 1999, Blows & Higgie 2003, McGuigan et al. 2005). This indicates that it is possible to break down genetic constraints (i.e. to evolve into a direction different from $g_{max}$), especially in the long term, and that the constraints themselves are subject to evolution (i.e. the orientation and magnitude of G may change with time; Lande 1976, Zeng 1988, Arnold 1992). The impermanence of genetic constraints is also supported by the vast diversity of organisms, even within populations.

### *Variation between individuals within populations*

Within population divergence, namely that between the sexes, offers a way to test the effects of genetic constraints on evolutionary responses. Evolution of sexual dimorphism (SD) involves a puzzle: sex-specific selection acts on genes that are shared by both sexes (Fisher 1930, 1931). In other words, there is a genetic correlation between the sexes ($r_{g(MF)}$), which is likely to slow down the evolution of SD (Lande 1980, Reeve & Fairbairn 2001, Fairbairn et al. 2007). Theoretically, in the extreme case, when genetic correlation between the sexes for a given trait is perfect ($r_{g(MF)} = 1$), SD should not evolve. However, even in the presence of high $r_{g(MF)}$, SD can still evolve if the sexes differ in amount of genetic variance for a trait in question (Lynch & Walsh 1998).

Simulations have shown that genetic correlations between the sexes constrain the evolution of sexual dimorphism much less than predicted by Lande's (1980) model (Reeve & Fairbairn 2001), which is built around the matrix of genetic variances and covariances between the sexes. Evidence from empirical studies is mixed. A recent meta-analysis of 66 dioecious plant and animal populations revealed a significant negative association between SD and $r_{g(MF)}$, while a survey of 68 studies on hermaphroditic plants revealed mixed results – the relationship between SD and $r_{g(MF)}$ depended on the species in question (Ashman & Majetic 2006). Studies involving enough traits so that the association between SD and can be tested on the traits within the study are few, and the results from them are also mixed (no correlation: e.g. Cowley et al. 1986, Cowley & Atchley 1988; negative correlation: Ashman 2003, Bonduriansky & Rowe 2005, McDaniel 2005, Fairbairn et al. 2007). The evidence thus indicates that the effect of genetic constraints on the evolution as inferred from sexual dimorphism is still unresolved.

Whether the genomic conflict between sexes imposes severe constraints on the evolution of SD or not, the driving force behind SD is differential selection in males and females. The differential selection between sexes does not have to be sexual selection in its original, strict sense – effects caused by the struggle for mates (Darwin 1871). Disruptive selection between sexes can stem from differences in habitat that lead to differential evolutionary responses from males and females. In fact, similar ecological conditions as those that lie behind adaptive divergence between species or populations can also drive the evolution of SD (Bolnick & Doebeli 2003). Indeed, the ecological differences between the sexes can be larger than those between species (Schoener 1967, 1969, Dayan et al. 1994). It has been suggested that SD and adaptive divergence are interwoven so that when there are no constraints for the evolution of SD, the likelihood of adaptive divergence is restricted (Bolnick & Doebeli 2003). It is thus imperative to consider the differences, ecological and/or genetic, between the sexes, when aiming to uncover the mechanisms behind adaptive divergence (e.g. Butler et al. 2007).

Sexual dimorphism provides an example of how divergence itself can be adaptive and reduce intraspecific competition within populations. Divergence between sexes can be thought of as a meta-trait, comprising of combined effects of a number of single traits, and thus it should evolve according to the same principles as any other trait. If divergence itself can evolve, it should comprise a heritable component. Indeed, sexual dimorphism of a variety of traits has been found to be heritable, but the estimates of heritability have been restricted to domestic animals or model species (e.g. Cowley et al. 1986, Chapuis et al. 1996, Mignon-Grasteau et al. 1998, David et al. 2003, but see Vandeputte et al. 2007). The published heritability estimates for SD are relatively low (Fig. 1), following the general pattern of decreasing heritability with increasing complexity of traits (Price & Schluter 1991, Merilä & Sheldon 1999).

Strong genetic correlations between the sexes can lead to the situation, where despite of sexual or disruptive selection, male and female phenotypes cannot reach their respective optima. Sexual dimorphism therefore provides one possible solution to the dilemma of how genetic variation is maintained in populations despite of directional selection. Theoretically, if natural selection is driving individuals towards a phenotype that is optimal in terms of fitness, variation around the optimal phenotype should in time diminish. Yet, genetic variation persists despite of strong directional selection (e.g. Barton & Turelli 1989), and even phenotypes that lie outside of the fitness optimum can be heritable. For instance, it has been shown that developmental instability, as inferred from fluctuating asymmetry, can include a heritable component of variance (Møller & Thornhill 1997).

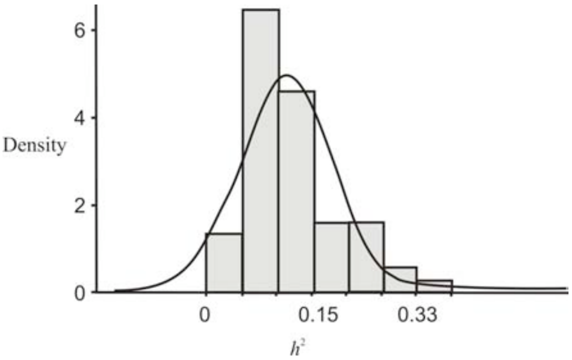

Figure 1. Distribution of 61 heritability estimates of sexual dimorphism reported in literature. The heritability estimates were extracted from Araripe et al. (2008), Buvanendran (1969), Chapuis et al. (1996), Cowley et al. (1986), David et al. (2003), Hanrahan & Eisen 1973, Hu et al. (1999), Le Bihan-Duval et al. (1998), Lester et al. (1989), Mignon-Grasteau et al. (1998, 1999, 2004), and Vandeputte et al. (2007).

### *Threespine stickleback as a model organism*

The threespine stickleback (*Gasterosteus aculeatus*) has a long history as a model species evolutionary biology. It has traditionally been the pet organism for behavioural ecology, to the extent that it has earned the nickname “white rat of ethology” (Huntingford 2003). Recently, threespine stickleback has become a prominent model in evolutionary genetic research, to the extent that the whole genome of the species has been sequenced.

The threespine stickleback is a small teleost abundant in marine, coastal and freshwater habitats throughout the Northern hemisphere. It is considered to form a species complex with a number of divergent populations (Bell & Foster 1994). The common name of threespine stickleback derives from three dorsal spines, of which the most posterior one is the shortest. There are also two robust pelvic spines that are supported by pelvic skeleton. The sides of the threespine stickleback are covered to varying extent by bony lateral plates that lie on top of myomeres (body segments). Bony armour is perhaps the most obvious character that varies among threespine stickleback populations across different habitats (Bell & Foster 1994). Bony armour is also the feature from which the species draws its scientific name *Gasterosteus* (*gaster* referring to stomach in Latin and *osseus* meaning bones or bone-like in Greek).

Threespine stickleback evolution is often characterised by divergence in bony armour. The reason being that the reduction in bony armour offers an astonishing example of parallel evolution. The reduction has been observed in numerous independent instances in different parts of the distribution range of the species (reviewed in Bell & Foster 1994). The main trend in distribution of the different lateral plate morphs is that ancestral marine morphs are fully plated, while reduction in the number of lateral plates is evident in landlocked freshwater populations (Bell & Foster 1994, Bell 2001). Reduction of the lateral plates can occur very rapidly on an evolutionary time scale, as has been recently reported by Bell *et al.* (2004). They demonstrated a reduction from fully plated to low plated morph in less than twenty years after colonisation of a freshwater lake. Pelvic girdle reduction is more rare than reduction in the lateral plate number – the complete loss of the pelvic girdle has been reported only in a few populations from recently deglaciated regions (Bell 1987). In evolutionary time scale however, the complete reduction of the pelvic structure in less than 10 000 years is fairly rapid (Shapiro *et al.* 2004). Evolution of other morphological aspects of threespine sticklebacks, such as body shape follows the patterns of bony armour evolution. Colonisation of freshwater habitats by the ancestral marine threespine sticklebacks has lead to repeated patterns of body shape divergence throughout the distribution range of the species (e.g. Walker 1997, Walker & Bell 2000, Kristjansson et al. 2002, Spoljaric & Reimchen 2007, Aguirre et al. 2008, Sharpe et al. 2008, Aguirre 2009).

The advances in the genetic tools available for threespine stickleback research together with the well-established knowledge of basic biology and ecology have lead to threespine sticklebacks becoming one of the leading model species for the study of genetic basis of adaptive divergence. Quantitative trait loci as well as individual genes that control divergence in quantitative traits have already been discovered (Peichel et al. 2001, Shapiro et al. 2004, Colosimo et al. 2005, Kimmel et al. 2005, Albert et al. 2008), and changes in the frequencies of individual alleles have been shown to be related to the colonisation of freshwaters by the marine ancestors (Barrett et al. 2008). The advent of increasingly sophisticated methods, such as microarrays for gene expression, is likely to result in an unprecedented increase in the resolution of studies targeting the genetic basis of adaptive divergence of threespine sticklebacks and further our understanding of evolutionary mechanisms to a completely new level.

## Aims of this thesis

The main aim of this thesis was to investigate the relative roles of genetic drift, natural selection and genetic constraints in the evolution of complex traits. More specifically, the aim was to find out how the three factors have contributed to the body shape and armour divergence of threespine sticklebacks.

The first aim of this thesis was to quantify the extent and patterns of morphological divergence in Fennoscandian threespine stickleback populations. The next aim was to find out whether the observed patterns of divergence were the result of natural selection or could be explained by genetic drift alone (I), and whether this result conformed with the general trends found in already published studies on the relationship between drift and selection (II). Additionally, since the estimates of quantitative trait divergence ($Q_{ST}$) in the first chapter were based on phenotypic data alone ($P_{ST}$), an additional aim was to explore whether there is a general trend in the published studies that the usage of $P_{ST}$ as a proxy for $Q_{ST}$ would result in overestimation of quantitative trait divergence.

Once the driving forces behind the morphological divergence were established, the aim shifted to uncovering the genetic basis of divergence. The aim of Chapter III was to investigate the genetic variation and covariation in body shape and armour traits, and based on these patterns deduce what would be the most likely directions of evolution in these traits. Constraints can have an effect on divergence at the population level, but also within populations in the form of genomic conflict between sexes. The aim of Chapter IV was to find out whether there are constraints imposed by the genomic conflict between the sexes in the ancestral population, and whether these possible constraints are reflected in the patterns of divergence in the derived populations. The aim of Chapter V was to go down one level, to variation within individuals, and to investigate the possibility that a trait reflecting developmental stability could express additive genetic variance. Once the relative roles of genetic drift, natural selection and G were established, it remained to test whether the observed patterns of divergence could be experimentally recreated (VI), using the knowledge on the mechanisms behind divergence (I, III). The aim of the final chapter was also to find out in detail how genetically correlated traits are affected by selection by a predator.

## Main results and discussion

Specific study questions and the main results of all the chapters are summarised in Table 1. Below I discuss these results and their relevance to the study of evolutionary biology of threespine sticklebacks, and evolution in general.

### *Relative roles of drift and selection in population divergence*

Prior to the present work (I), and the pioneering phylogenetic study by Mäkinen et al. (2006), the extent and patterns of threespine stickleback divergence in Fennoscandia were largely unknown. The results of Chapter I show that body shape divergence among Fennoscandian threespine stickleback populations largely follow the patterns of body shape divergence observed in other parts of the distribution range of the species (e.g. Walker 1997, Walker & Bell 2000, Spoljaric & Reimchen 2007, Aguirre et al. 2008, Aguirre 2009). The largest differentiation was found between threespine sticklebacks from marine and freshwater habitats, but body shape also differed among the lake populations depending on the size of the lake (I). Among the lake populations, the patterns of differentiation in neutral genetic markers were similar to those in body shape differentiation, suggesting that the extent of body shape divergence of Fennoscandian freshwater threespine sticklebacks is what could be expected under drift alone. However, overall and between habitats, phenotypic differentiation ($P_{ST}$) exceeded the differentiation in neutral markers ($F_{ST}$; Fig. 2), suggesting that directional natural selection has been the main factor influencing divergence among the sea populations, as well as across habitats (Fig. 2). Among the sea populations, despite virtually nonexistent neutral genetic divergence ($F_{ST}$), clear phenotypic divergence was present ($P_{ST}$; Fig. 2), providing evidence that adaptive divergence can take place even in the presence of significant levels of gene flow (Hendry et al. 2002, Hendry & Taylor 2004, Hemmer-Hansen et al. 2007).

Estimation of quantitative trait differentiation using only phenotypic data might result in an overestimation of population divergence due to inclusion of environmental and non-additive genetic effects in the $P_{ST}$ estimates (e.g. Merilä & Crnokrak 2001, Lee & Frost 2002). In our case however, gross overestimation is unlikely for a number of reasons. First, the conclusions did not change even when assuming that half of the phenotypic variation was additive ($h^2$ = 0.5; Fig. 2). Second, the aspects of body shape showing the most divergence have been demonstrated to have genetic basis (McPhail 1984, Albert et al. 2008, III). Finally, according to the meta-analysis of the published comparisons of quantitative trait and neutral genetic divergence, studies based on data from the wild do not tend to yield higher estimates than $Q_{ST}$ studies based on common garden data (II). In fact, the meta-analysis also revealed that there were no significant differences in the $Q_{ST}$ estimates for different trait types (*viz.* morphological, life history or behavioural), contrary to what could have been expected based on how traits with different genetic basis (*i.e.* lower $h^2$ in traits more closely related to fitness; Mousseau & Roff 1987,

Table 1. Summary of the main study questions and results of the six chapters included in this thesis.

| | Main study questions | Main results |
|---|---|---|
| I | What is the extent of body shape divergence in the Fennoscandian threespine stickleback populations? | The patterns of body shape divergence follow those found elsewhere in the distribution area of threespine sticklebacks, largest differentiation occurring between marine and freshwater populations. |
| | Can the observed patterns of divergence be explained by genetic drift alone, or are they the result of natural selection? | Natural selection has been a predominant force behind the observed patterns of body shape divergence, although genetic drift may have contributed to the divergence, especially in the small lake populations. |
| II | Does natural selection have a predominant role over genetic drift in the general patterns of quantitative trait divergence? | Yes. Natural selection has a predominant role as a cause of differentiation in quantitative traits. |
| | Does divergence in neutral markers reflect divergence in quantitative traits? | No. Conclusions on quantitative trait divergence should not be made based solely on divergence in neutral genetic markers. This is especially important in cases in which neutral genetic divergence is low |
| III | What is the direction of maximum additive genetic variance ($g_{max}$) in threespine body shape? | Direction of $g_{max}$ reflects the divergence observed in wild populations. |
| | Are the directions of additive genetic and phenotypic variance correlated? | Yes. Directions of maximum genetic and phenotypic variance are correlated. |
| | Are genetic correlations imposing possible constraints on body shape and armour evolution? | Body shape is genetically correlated with armour traits, which should be taken into account when identifying selective factors responsible for evolution of single traits |
| IV | Is there a genomic conflict between male and female threespine sticklebacks and is it imposing possible constraints on the evolution of sexual dimorphism? | There is a genomic conflict between males and females, as inferred from the high genetic correlations in body shape and armour traits between the sexes. |
| | Are the possible constraints reflected in the patterns of sexual dimorphism observed in wild populations? | The effect of genomic conflict is not reflected in the patterns of sexual dimorphism in derived threespine stickleback populations. |
| V | Is there a heritable component to the variance in fluctuating asymmetry of lateral plates – a trait reflecting developmental stability? | Yes. This is the first study to find substantial amounts of additive genetic variance in fluctuating asymmetry with appropriate methods and experimental design. |
| VI | What are the targets and patterns of predator induced selection in threespine stickleback morphology? | Body size and shape, specifically the length of the caudal peduncle, are both affected by selection by predators. |
| | Can different antipredator tactics in pelagic and benthic habitats explain the reduction in the number of lateral plates? | Differing antipredator tactics in pelagic and benthic habitats can be responsible for the lateral plate number reduction during freshwater colonisation of threespine sticklebacks. |

Merilä & Sheldon 1999), and the results of earlier meta-analysis of studies comparing $Q_{ST}$ and $F_{ST}$ (Merilä & Crnokrak 2001). The general patterns in studies comparing $Q_{ST}$ and $F_{ST}$ estimates suggest that natural selection plays a predominant role in quantitative trait differentiation. It must be noted though that this is the case for those systems that are likely to be known *a priori* to exhibit pronounced phenotypic divergence – they are possibly not a representative sample of systems in respect to degree of population divergence.

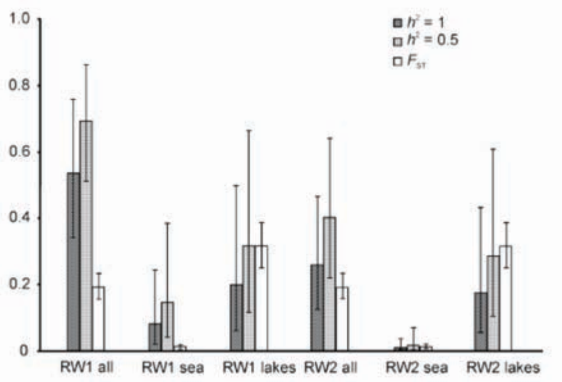

Figure 2. $P_{ST}$ values for body shape (the first two principal components) assuming two different heritability ($h^2$) values along with $F_{ST}$ values. All values are with 95 % Bayesian confidence intervals. Variance in shape is adjusted for variance in sex and body size.

There has been some controversy around the issue whether neutral and quantitative genetic estimates are correlated or not (Merilä & Crnokrak, 2001; Crnokrak & Merilä, 2002; Latta & McKay, 2002; McKay & Latta, 2002). In theory, one index of divergence should not reflect the other (Pearman 2001, McKay & Latta 2002), but certain demographic scenarios, such as habitat fragmentation and reduced gene flow, might influence both $Q_{ST}$ and $F_{ST}$ in a similar fashion (Frankham & Weber 2000). This might be one explanation for the lack of difference in $P_{ST}$ and $F_{ST}$ estimates among Fennoscandian freshwater populations of threespine sticklebacks (I). The meta-analysis revealed a positive correlation between $Q_{ST}$ and $F_{ST}$ across studies, although with small $F_{ST}$ values, $Q_{ST}$ values were highly variable (II). This implies that extreme caution should be exercised before making any conclusions on quantitative genetic differentiation based solely on neutral marker differentiation (e.g. Hendry 2002).

### *Within population variance and population divergence*

Genetic variation and covariation have a fundamental role in evolutionary change. Without additive genetic variation, there will be no evolution, while covariation between traits means that change in one trait could induce a change in the other. The possible constraining role of the patterns of covariation between traits has lead to the prediction that evolution is most likely to take place along the line of least resistance, i.e. to the direction of maximum additive genetic variance ($g_{max}$; Schluter 1996). It has been fairly well established that threespine stickleback morphological divergence follows similar patterns all over the distribution range of the species (for armour traits: see reviews by Bell & Foster 1994, Bell 2001, Östlund-Nilsson et al. 2007; for body shape: e.g. Walker 1997, Walker & Bell 2000, Kristjansson et al. 2002, Spoljaric & Reimchen 2007, Sharpe et al. 2008, Aguirre et al. 2008, Aguirre 2009, I). If Schluter's (1996) predictions on the evolution along $g_{max}$ are correct, the divergence along $g_{max}$ of an ancestral population of threespine sticklebacks should be similar to that observed in the wild populations. Chapter III shows that this is the case for threespine stickleback body shape. Body shape divergence in the wild (Walker 1997, Walker & Bell 2000, Kristjansson et al. 2002, Spoljaric &

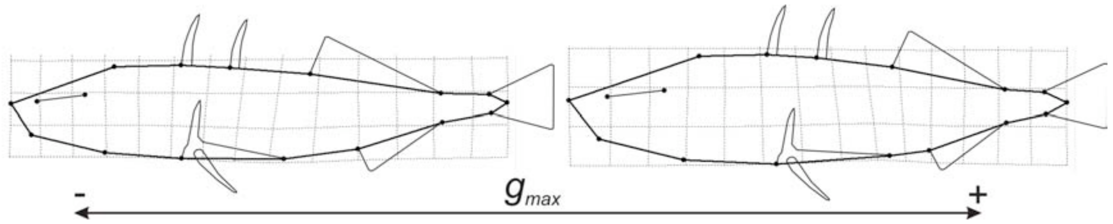

Figure 3. Illustration of body shape divergence along the major axis of genetic variation.

Reimchen 2007, Sharpe et al. 2008, Aguirre et al. 2008, Aguirre 2009, I) and along the $g_{max}$ of the putative ancestral population follow the same patterns; at one end there are threespine sticklebacks with deep bodies, short caudal peduncles, large heads and posterior positioned dorsal spines, pelvis and median fins – typical deep-bodied sticklebacks, while at the other end there are threespine sticklebacks with narrow bodies, long caudal peduncles, small heads and anterior positioned dorsal spines, pelvis and median fins – typical streamlined sticklebacks (Fig. 3).

Directions of maximum additive genetic and phenotypic variance were also correlated, which implies that phenotypic divergence is a fair surrogate for genetic divergence in threespine stickleback body shape (III). This is concordant with results of studies on other taxa and traits (Cheverud 1988, 1996, Roff 1995, Reusch & Blanckenhorn 1998, Waitt & Levin 1998, Reale & Festa-Bianchet 2000, House & Simmons 2005, Åkesson et al. 2007, de Oliveira et al. 2009). The results of Chapter III also show that there are strong negative as well as strong positive genetic correlations between body shape and armour traits. Therefore, disentangling the selective agents responsible for the evolution of any single trait in threespine sticklebacks might be difficult – what may appear to be a direct response to selection on a particular trait (e.g. length of the pelvic girdle) might in fact be a correlated response to selection on another trait.

Although evolution of threespine stickleback body shape appears to take course along the path dictated by genetic covariances and correlations, this was not the case with sexual dimorphism (IV). Genetic correlations between the sexes ($r_{g(MF)}$) were high and the lines of least resistance to evolution ($g_{max}$) had similar directions in males and females, but there was no association between $r_{g(MF)}$ of the ancestral population and the degree of sexual dimorphism in the derived wild populations. Majority of evidence from published studies points to the direction that $r_{g(MF)}$ and sexual dimorphism are negatively correlated (Poissant et al. 2010), but the results of Chapter IV add to the few exceptions where no such association has been found (Cowley et al. 1986, Cowley & Atchley 1988, Ashman & Majetic 2006). The lack of association between $r_{g(MF)}$ and sexual dimorphism also indicates that in threespine sticklebacks, sex-specific selection pressures are strong enough to overcome the genetic constraints, and/or that the sex-limited gene expression can be regulated with relative ease.

Genomic conflict between the sexes is one possible factor that maintains genetic variation despite of directional selection, which theoretically depletes genetic variation. Because of the genetic correlations between male and female traits, it is more difficult for the traits to reach their optimum in terms of fitness. This is also one possible way in which traits indicative of developmental stability, such as fluctuating asymmetry, can have an additive genetic component of variance. Traditionally, heritability estimates for fluctuating asymmetry have been very low (Leamy 1997, Fuller & Houle 2003), but the estimates have usually suffered from difficulties affecting their accuracy (e.g. Merilä & Björklund 1995, Whitlock 1996, Björklund & Merilä 1997, van Dongen 2006). Chapter V is the first study that has found a substantial amount of additive genetic variance in fluctuating asymmetry ($h^2 = 0.24$) using appropriate methods and experimental design. Selection by predators has been found to favour symmetric threespine sticklebacks (Moodie & Reimchen 1976, Bergstrom & Reimchen 2003), implying that selection on fluctuating asymmetry of lateral plates may have important evolutionary consequences.

### *The role of selection in adaptive divergence*

With the predominant role of selection in morphological divergence of threespine sticklebacks between marine and freshwater habitats established (I), as well as the possible constraints imposed by the genetic variance-covariance matrix, G (III), the final component missing from the breeder's equation was the actual effect of selection on the divergence. Although a number of hypotheses on the effects of abiotic and biotic factors have been advanced to explain the divergence in armour (reviewed by Bell 1995, Reimchen 2000, Bell 2001; see also Marchinko & Schluter 2007, Barrett et al. 2008), as well as in body shape (e.g. Walker 1997, Walker & Bell 2000, Spoljaric & Reimchen 2007, Aguirre et al. 2008, Aguirre 2009), the effect of predation is still included in most of the hypotheses (for armour see reviews by Reimchen 1994, Bell 2001). The positive effect of predation on presence of lateral plates has been experimentally shown (Moodie et al. 1973, Bell & Haglund 1978, Reimchen 1992, 2000, Vamosi 2002), but the effects of predation on the reduction of plate number has not been experimentally tested, although it has been hypothesised that different antipredation strategies in different habitats lie behind the reduction in lateral plates (Reimchen 1992). Likewise, the role of predation in body shape differentiation of threespine sticklebacks has not been experimentally tested.

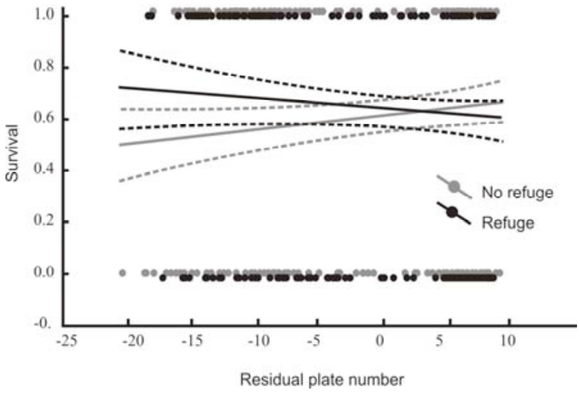

Figure 4. Survival probability (relative fitness) of threespine sticklebacks as a function of number of lateral plates (corrected for body size) in the experiments with (black dots and line) and without (grey dots and line) refuge. Dotted lines denote the 95% confidence intervals.

Chapter VI provides direct experimental support for Reimchen's (1992) hypothesis that different antipredatory tactics in pelagic and benthic habitats could be responsible for the lateral plate number reduction during freshwater colonisation in threespine sticklebacks. In habitats with refuge, selection favours individuals with low number of lateral plates, while fully plated individuals have a selective advantage in habitats without shelter (Fig. 4). Differences in body shape also have an effect on survival in the presence of predators (VI). Swimming performance related aspects of body shape, such as the length of the caudal peduncle and body depth (Webb 1982, 1984) appear to be the most important predictors of survival from predators. Taking into account the genetic correlations between body shape and armour traits revealed that spine lengths also have an effect on survival, as expected (Hoogland et al. 1957, Vamosi 2002). It is notable that this effect was not evident when analysing the effects of different traits individually. Therefore, it is imperative to consider genetic correlations between traits when drawing conclusions on the effects of selection on single traits (Lande & Arnold 1983).

## Conclusions and future directions

I have demonstrated how evolution of complex traits, investigated at different levels of resolution can produce a holistic picture of the evolutionary processes. By following the theoretical framework for multivariate trait evolution ($\Delta z = GP^{-1}s$; Lande 1979, Lande & Arnold 1983), I have shown how evolutionary mechanisms can influence adaptive divergence from the level of individuals to populations. Body shape divergence of threespine sticklebacks between populations from marine and freshwater habitats is clearly driven by natural selection, while genetic drift may play significant role in more fine scale divergence, between populations of isolated freshwater habitats. Body shape divergence along the most likely direction of evolution ($g_{max}$; Schluter 1996) in the ancestral marine threespine sticklebacks is similar to that observed in wild populations (e.g. Walker & Bell 2000, Spoljaric & Reimchen 2007, I), indicating that genetic variances and covariances (G) can significantly bias the patterns of evolution.

Genetic conflict, as imposed by high genetic correlations between males and females, appears to be resolved in threespine sticklebacks. Ancestral genetic correlations are not reflected in the patterns of sexual dimorphism in the derived wild populations. The patterns of ancestral sexual dimorphism in body shape reflect the patterns of body shape differentiation observed in threespine stickleback marine-freshwater transition (e.g. Walker & Bell 2000, Spoljaric & Reimchen 2007, I), indicating that ancestral sexual dimorphism could have played a significant role in the adaptive divergence of threespine sticklebacks. Variation in traits within individuals can also diverge and help maintaining genetic variation in a population under directional selection. The significant additive genetic variance component of fluctuating asymmetry provides a demonstration of how phenotypes that are assumed to be outside their fitness optimum can be inherited, and thus, have an influence on evolution.

Finally, I have shown that the patterns of evolution seen in nature can be experimentally recreated to tease out the effects of natural selection on adaptive divergence in detail. I have also provided a demonstration of how important it is to take into account the correlative nature of traits, when interpreting the effects of natural selection. The effects of selection on some traits might be hidden because of selection on genetically correlated traits. Threespine stickleback evolution is characterised by rapid reduction in bony armour, and I have shown how different antipredatory strategies in pelagic and benthic habitats could be responsible for the lateral plate number reduction during freshwater colonisation. Definitive tests of the effect of predation on the reduction of lateral plates have been thus far lacking, although the effect of marine to freshwater transition on individual genes that affect the number of lateral plates have recently been demonstrated (Barrett et al. 2008). The future challenge lies in trying to establish a basis for adaptive divergence of complex traits, such as body shape, at the level of genes. It is likely though, that instead of changes in genes themselves, changes in gene regulation could play an important role in adaptive divergence of complex traits. For example, changes in gene regulation are more readily achieved than actual changes in the genes themselves in cichlid fishes (Roberts et al. 2009). The recent methodological advances in molecular genetics bring promise on opportunities to take the study of adaptive divergence into a completely new level, especially in threespine sticklebacks with completely sequenced genome.

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