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Strong and consistent natural selection associated with armour reduction in sticklebacks

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Abstract

Measuring the strength of natural selection is tremendously important in evolutionary biology, but remains a challenging task. In this work, we analyse the characteristics of selection for a morphological change (lateral-plate reduction) in the threespine stickleback *Gasterosteus aculeatus*. Adaptation to freshwater, leading with the reduction or loss of the bony lateral armour, has occurred in parallel on numerous occasions in this species. Completely-plated and low-plated sticklebacks were introduced into a pond, and the phenotypic changes were tracked for 20 years. Fish from the last generation were genotyped for the *Ectodysplasin-A* (*Eda*) locus, the major gene involved in armour development. We found a strong fitness advantage for the freshwater-type fish (on average, 20% fitness advantage for the freshwater morph, and 92% for the freshwater genotype). The trend is best explained by assuming that this fitness advantage is maximum at the beginning of the invasion and decreases with time. Such fitness differences provide a quantifiable example of rapid selection-driven phenotypic evolution associated with environmental change in a natural population.

Keywords: adaptive evolution, *Ectodysplasin-A*, *Gasterosteus aculeatus*, maximum likelihood, population genetics, selection response

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Introduction

Measuring the strength and the characteristics of natural selection in populations remains a difficult task (Endler 1986; Schluter 1988; Kingsolver *et al.* 2001; Hereford *et al.* 2004; Siepielski *et al.* 2009). Natural selection generally involves multiple trade-offs between various life history traits, and the characters on which selection acts are often underlain by complex genetic architectures (Rogers & Bernatchez 2007). Moreover, environmental effects influencing natural selection may change frequently, so that it is often difficult to quantify the different factors influencing the phenotypic changes observed in the wild.

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Recent work has suggested that the threespine stickleback, *Gasterosteus aculeatus*, provides a good model for studying natural selection in wild populations (Gibson 2005; Barrett 2010). The stickleback is originally a marine fish, but has repeatedly colonized freshwater lakes (Bell 2001; Lucek *et al.* 2010; Berner *et al.* 2010). Convergent adaptation to new freshwater environments is associated with consistent phenotypic changes, such as the reduction or loss of lateral armour. Marine threespine sticklebacks are protected by up to seventy lateral calcified plates; the number of plates is greatly reduced in many sticklebacks living in lake and river environments (Bell *et al.* 2004). This system is particularly convenient to use as a model of adaptation for several reasons, including that (i) the expression of lateral plates is based on a quite simple, almost Mendelian genetic architecture, involving a major-effect locus (Cresko *et al.* 2004; Colosimo *et al.* 2004, 2005); (ii) adaptation appears to

occur systematically from standing genetic variation (marine fish carry the freshwater genotype with a frequency of around 1%), so that the genotype at this main locus can be tracked with genetic markers (Barrett *et al.* 2008); and (iii) the armour loss is highly repeatable and apparently fast (Bell 2001; Bell *et al.* 2004; Lucek *et al.* 2010).

In open systems, gene flow between river, lake and marine stickleback populations can lead to spatially complex genetic and selection patterns (Kitano *et al.* 2008; Bolnick *et al.* 2009). Moreover, the direction of selection is not as stable as expected and may be reversible, either because of environmental changes (Kitano *et al.* 2008) or because of different selection pressures at different life stages (Barrett *et al.* 2008, 2009). The mechanisms underlying the long-term phenotypic changes observed when sticklebacks invade freshwater are thus not fully clear.

In this contribution, we aim to deepen our knowledge about this model adaptation by analysing the dynamics of the selection process. From the recording of phenotypic and genetic changes over 20 years in a stickleback population, we obtained insights into (i) the strength of selection, i.e. the difference in fitness between freshwater- and marine-type individuals, (ii) the properties and the stability of natural selection over time, and (iii) the association between fitness, lateral plate morph and the underlying genotype at the major gene *Ectodysplasin-A* (*Eda*).

Materials and methods

Experimental setting

In 1987, 250 low-plated and 250 completely plated sticklebacks, all of them already adapted to freshwater environments, were introduced in the Nygaardspark's 500-m^2 pond in Bergen (Norway), which is also inhabited by Crucian carps (*Carassius carassius* L.). Predation by birds or fish is expected to be very limited or absent, and the density of macroinvertebrates was low. The artificial pond is about 100 years old, and sticklebacks were absent before 1987 (trapping in 1986 was unsuccessful). The initial 250 low-plated fish were sampled in the lake Eikelandsvatn (Bergen), where the population is almost monomorphic for low-plated morphs. The 250 completely plated fish come from the lake Myrdalsvatn (Bergen), in which all fish are completely plated. Myrdalsvatn is indeed one of the few freshwater environments in Norway hosting completely plated sticklebacks; its colonization is probably recent and artificial (the altitude of the lake being too high for natural migration of marine sticklebacks). The most obvious explanation for the unexpected phenotype in Myrdalsvatn is thus the absence of low-morph alleles in a small founder population.

Every year between 1988 and 1993, 100–200 sticklebacks were sampled in Nygaardspark pond with a plexiglass trap, fish were collected throughout the summer season and pooled. Three additional samples were collected in 2006, 2007 and 2008. Fish were killed, fixed in ethanol and classified according to the three classical morphs (low, partially and completely plated). The scoring of plate morphs and plate numbers was performed without staining; fixation in ethanol leads to a slight dehydration of the skin and makes the plate scoring easy using a dissecting microscope. Discrimination between morphs was based on the criteria proposed by Ziuganov (1983): fish with 10 or less plates on one side (left side) were classified as low plated, while fish having more than 21 anterior plates on this side (excluding keel plates) were considered as completely plated; intermediates are partially plated. These criteria are slightly different from those used e.g. by Bell *et al.* (2004), in which the presence or absence of keel plates discriminates low and partial morphs. Nevertheless, both classifications largely overlap (only 33 differences of 319 measured fish in our 2008 sample, Table S1, Supporting information). In addition, 151 fish from the 2008 sample (approximately 50 of each morph), as well as 32 fish from Eikelandsvatn and 32 from Myrdalsvatn, were genotyped for the *Stn381* and *Stn382* markers. Both are located in two different introns of the gene *Ectodysplasin-A* (*Eda*), known to be the major genetic factor in lateral-plate expression (Colosimo *et al.* 2005). Alleles were labelled according to Colosimo *et al.* (2005), allele *A* corresponds to the marine form and allele *a* to the low-plated, freshwater populations.

Independent data from Loberg lake, Alaska

A 12-year time series with three morphs (instead of the five reported) was reconstructed from table 1 in Bell *et al.* (2004). Our population genetics models were fitted to the data, with two adjustments: (i) sample size was considered to be large and constant ($N = 1000$ every year), otherwise the model ignores the early part of the time series, in which the sample size was two orders of magnitude smaller than the last years, and (ii) the parameters describing the genetic architecture (morph conditional probabilities) were adapted to reflect a difference in the genetic background of the fish compared to Nygaardspark pond. Indeed, when low and complete morphs are at equal frequencies (which is a comparable stage in both time series), there are at least 30% of partially plated individuals in Nygaardspark pond, but only

15% of fish are partially plated in Loberg lake. As it is known that several minor genes can influence the plate morph (Colosimo *et al.* 2004), this difference was attributed to the genetic background of both populations, and all probabilities of being partially plated (originally estimated from Nygaardspark pond) were divided by two for the Loberg lake analysis. When these probabilities are not adjusted, the fitness estimate of the low morph is barely affected, but the fitness of partially plated fish is divided by two (the model explains the lack of intermediate morphs by strong selection against those, the fit remaining bad, 200 AIC units behind the adjusted data).

Genotyping

DNA was extracted from the pectoral fin using a standard proteinase K phenol–chloroform protocol (Sambrook *et al.* 1989). Two markers were amplified to determine the lateral-plate morph-specific genotypes at the *Eda* gene; *Stn381* (forward: 5′-CAC GGA CTT ACA CCA CAA CG -3′, reverse: 5′- ATT CGA GGG TTC AGC TCT GG -3′, revealed by fluorescently labelled primers) and *Stn382* (forward: 5′- CCC TTA GAG AAT TTC CTA GCA G -3′, reverse: 5′- CTT GTC CCG GAT CAT ACG C -3′, visualized on an agarose gel) are located within intron 6 and 1 in the *Eda* gene, respectively (Colosimo *et al.* 2005; Raeymaekers *et al.* 2007; Kitano *et al.* 2008; Marchinko 2009; Barrett *et al.* 2008, 2009).

Stn381. The PCR amplification was perfomed in a multiplex along with four other loci (not used in the current study) in a 25-μL reaction volume containing 15 ng of genomic DNA, two units of Taq polymerase, 2.5 μL of 10×reaction buffer (10 mM Tris–HCL (pH 9), 50 mM KCl, 0.1% Triton X-100), 1.8 mM $MgCl_2$, 0.2 mM dNTPs, 4 pmol each of the *Stn381* primers (and 6–20 pmol of the remaining four primer sets) and 9.3 μL dd H_2O. The PCR was performed on a T1 Thermocycler (Biometra, Germany) machine with an initial denaturation for 3 min at 95 °C, followed by one cycle of: (30 s at 95 °C, 30 s at 59 °C, 30 s at 72 °C), five cycles of (30 s at 94 °C, 30 s at 59 °C, 30 s at 72 °C), 35 cycles of (30 s at 90 °C, 30 s at 60 °C, 30 s at 72 °C), followed by 30 s at 72 °C and final extension of 10 min at 72 °C, pausing at 6 °C. Electrophoresis was conducted on an ABI 3100 automated sequencer using the size standard Genescan-500 LIZ (Applied Biosystems, Foster City, California). PCR products were diluted for electrophoresis in groups of loci based on their molecular mass and attached fluorescent label. One microlitre of diluted PCR products was added to 10 μL of deionized formamide and 0.15 μL of the size standard Genescan-500 LIZ (Applied Biosystems). Fluorescent DNA fragments were analysed using GENESCAN and GENOTYPER 3.7 (Applied Biosystems). *Stn381* displays two alleles with respectively 170 and 188 bp, easily scored.

Stn382. PCR simplex amplification of *Stn382* was performed in an 15 μL reaction volume containing 9 ng genomic DNA, one unit of Taq polymerase, 1.5-μL of 10×reaction buffer [10 mM Tris–HCL (pH 9), 50 mM KCl, 0.1% Triton X-100], 1.8 mM $MgCl_2$, 0.13 mM dNTPs, 33 pmol of each of the primers and 6.6 μL dd H_2O. The PCR was performed as for *Stn381*. To visualize the amplified alleles, a mix of 7 μL of the PCR product together with 3 μL of BB blue and 2 μL of H_2O was run on a 1.0% agarose gel with 100-bp ladder (Invitrogen cat # 15628-019) at 100 mV for 1 h. *Stn382* displays two alleles with respectively 158 and 218 bp, which can be easily scored.

Population genetics models

We estimated the strength of selection on lateral-plate morphs based on a population genetics model developed by Le Rouzic *et al.* (2010) (see also Wright & Dobzhansky 1946; DuMouchel & Anderson 1968; Prout 1969 for similar models). Because the link between *Eda* and the lateral plate morph is not perfect, two families of models were considered: either selection acts on the genotype (the 'genotype-selection model': the fitness of a fish depends on its *Eda* genotype, whatever its morph) or selection acts on the phenotypic morph ('morph-selection model'). Models in which the selection strength was allowed to vary (the fitness advantage of the freshwater morph or genotype being higher when the freshwater forms are rare) were also considered (frequency-dependent selection models).

The population size and its dynamics being unknown (unlike in e.g. Wilson 1980; O'Hara 2005), infinite-size population models were used, and generations were considered as nonoverlapping (if generations are actually overlapping, fitness differences will be underestimated, so this hypothesis is conservative). Arbitrarily, the marine morph/genotype has been chosen as a reference and its fitness is always 1. The whole time series can be reconstructed from a few parameters: p_0, the initial allelic frequency, and two fitnesses (w_L and w_P for the morph-selection model, and w_{aa} w_{Aa} for the genotype-selection model).

General setting. Assuming random mating, the genotypic frequencies before selection at generation t follow Hardy–Weinberg frequencies, i.e. $f_{aa_t} = p_t^2$, $f_{Aa_t} = 2p_t(1-p_t)$, and $f_{AA_t} = (1-p_t)^2$, where p_t is the frequency

of allele *a*. The genetic empirical data show that the association between the marker genotype (expected to reflect the *Eda* genotype) and the phenotypic morph is not perfect, so that the frequencies of the three morphs *L*, *P* and *C* (for low, partially and completely plated) can be written as $f_{m_t} = \sum_g \mathbf{P}_c(m \mid g) \cdot f_{g_t}$, where m stands for any morph ($m \in \{L,P,C\}$) and g for any possible genotype ($g \in \{aa,Aa,AA\}$), f_{g_t} being the frequency of genotype g at generation t before selection.

The conditional probabilities marked with a 'c' are assumed to be constant, because they describe the genetic architecture (i.e. the probabilities for a given genotype to produce specific morphs). These probabilities were estimated from the 2008 population (Table S4, Supporting information).

Genotype-selection model. Let w_{aa} be the fitness of genotype *aa*, measured relative to the marine genotype *AA* (i.e. $w_{aa} = W_{aa}/W_{AA}$, where W stands for the absolute fitness). In the same way, $w_{Aa} = W_{Aa}/W_{AA}$, and by definition, $w_{AA} = 1$. Classically, the frequency of genotype g after selection is thus $f'_{g_t} = f_{g_t} w_g / \bar{w}_t$, where $\bar{w}_t = \sum_g f_{g_t} w_g$. The frequency of morph m after selection can also be evaluated as: $f'_{m_t} = \sum_g f'_{g_t} \mathbf{P}_c(m|g)$. The allelic frequency after selection is $p'_t = f'_{aa_t} + (1/2) f'_{Aa_t}$, and genotypic frequencies at the next generation (before selection) are $f_{aa_{t+1}} = p'^2_t$, $f_{Aa_{t+1}} = 2p'_t(1-p'_t)$ and $f_{AA_{t+1}} = (1-p'_t)^2$.

Morph-selection model. The morph fitnesses are defined in the same way as the genotypic fitnesses, i.e. $w_L = W_L/W_C$, $w_P = W_P/W_C$, and $w_C = 1$. After selection, the morph frequencies are $f'_m = w_m f_{m_t} / \bar{w}_t$, with $\bar{w}_t = \sum_m f_{m_t} w_m$. Assuming that selection targets the morphs directly, there is no selection within each morph, and intra-morph genotypic frequencies are identical before and after selection: $f'_{g_t} = \sum_m f'_{m_t} \mathbf{P}(g|m)_t$. The conditional probabilities can be calculated assuming constant genetic architectures, as $\mathbf{P}(g \mid m)_t = f_{g_t} \mathbf{P}_c(m \mid g) / f_{m_t}$.

In the same way as for the direct selection on the genotypes, the allelic frequency after selection is $p'_t = f'_{aa_t} + (1/2) f'_{Aa_t}$, and genotypic frequencies at the next generation can be predicted assuming random mating.

Frequency-dependent selection. The model predicts that the fitness of the freshwater morph depends on the frequency of this morph. The model is thus equivalent to the direct selection of phenotypic morphs, except that $w_{L_t} = w_{L_0} \cdot e^{-k \cdot f_{L_t}}$. In this setting, w_{L_0} is the fitness of the low morph when their frequency tends towards 0, and k is a constant featuring the strength of frequency-dependent selection. If $k = 0$, there is no frequency dependency (w_L is constant and equals to w_{L_0}), if $k > 0$, the fitness of the low morph decreases when its frequency increases, and if $k < 0$, the fitness increases when the morph becomes more common (the rare is disadvantaged). For simplicity, only one morph was considered as directly affected by frequency-dependent selection, the ratio between fitnesses of the two other morphs (complete and partial) was constant. Frequency-dependent selection on genotypes was modelled in a similar way, by assuming that $w_{aa_t} = w_{aa_0} \cdot e^{-k \cdot f_{aa_t}}$.

Implementation. The parameters of the models were estimated from the data by maximum likelihood. Assuming that the population size in the pond is much larger than the sample size, genetic drift can be neglected relative to sampling effects (the consequences of this assumption were investigated, see Appendix S1, Supporting information). The probability of observing exactly L_t low plated, P_t partially plated and C_t completely plated fish among N_t sampled sticklebacks at generation t, supposing that the sampling occurs after selection, follows a multinomial distribution $\mathcal{M}(L_t, P_t, C_t | f'_{L_t}, f'_{P_t}, f'_{F_t}, N_t)$, where $f'_{m_t}(p_0, w_L, w_P)$, written f'_{m_t} for simplicity, represents the expected frequency after selection of morph m at generation t. The likelihood of the whole time series is:

$$L(p_0, w_L, w_P | data) = \prod_{t \in T} \mathcal{M}(L_t, P_t, C_t | f'_{L_t}, f'_{P_t}, f'_{F_t}, N_t),$$

where T stands for the years available in the time series.

Our models are significantly more complex than earlier ones (e.g. Wright & Dobzhansky 1946; DuMouchel & Anderson 1968), and we maximized the likelihood function numerically. The model was implemented as a set of functions in R, and convergence (through the mle wrapper for the optim function) was generally unproblematic with the default settings. Before fitting, fitnesses were log transformed, and the initial allelic frequency was logit transformed, so that all optimized parameters are defined beween $-\infty$ and $+\infty$. Confidence intervals were computed from the profile likelihood, before being back transformed to the original scale. The statistical properties of the model were assessed by simulation (Appendix S1, Supporting information).

Results

Phenotypic changes

The phenotype was scored nine times over the 20 years of the experiment (Table 1). In addition, 151 fish from the 2008 sample were genotyped and their number of lateral plates was counted. The relation between the morphs and the number of lateral plates is illustrated in Fig. 1(a). The trimodal distribution confirms the pres-

Table 1 Number of completely plated, partially-plated and low-plated morphs sampled in the Nygaardspark pond between 1988 and 2008. The first line (1987) corresponds to the 500 fish introduced (not included in the analysis); 151 fish from the last sample (2008) were genotyped at the *Eda* locus

Year	Complete	Partial	Low
1987	250	0	250
1988	134	19	2
1989	111	8	6
1990	105	25	7
1991	86	25	23
1992	116	52	53
1993	134	53	51
2006	40	41	43
2007	46	44	58
2008	107	83	129

ence of three disctinct phenotypic morphs and justifies the analysis based on discrete categories instead of considering the lateral-plate number as Gaussian-distributed quantitative character.

Although an equal number of low- and completely plated sticklebacks were introduced in 1987, most fish sampled in 1988 were completely plated, suggesting an unexpected decrease in the frequency of the supposedly adaptive low-plated freshwater phenotype. Because this accident can hardly be explained by natural selection on the lateral armour, the first generation was discarded in further analyses. After 1988, however, the time series follow a regular trend in favour of the low-plated morph. Between 1988 and 2008, the completely plated (marine) morph decreased from 86% to 34%, while the low-plated phenotype increased from 1% to 40%. Overall, in 20 years, evolution drove the pond population more than half way from an almost monomorphic completely plated population towards a low-plated state.

Genotype-phenotype association

Lateral-plate expression is known to be influenced by a major quantitative trait locus (Colosimo *et al.* 2004), which has been located in the *Ectodysplasin-A* gene (*Eda*) (Colosimo *et al.* 2005). However, the relationship between the *Eda* genotype and the phenotypic morph is not one-to-one; genotyping the fish for the *Eda* locus thus complements the phenotypic measurements. More than 200 fish sampled in 2008 (151 from the pond population and 32 from each parental lake) were genotyped for two markers, *Stn381* and *Stn382*, located in introns of *Eda* (Colosimo *et al.* 2005). Contrary to the expectation, both markers gave different results (Tables S2, S3 and S4, Supporting information). *Stn381* displays a large deficit in heterozygotes and thus appeared to be less reliable. Thirty-nine fish were regenotyped to discard tagging or genotyping errors, and the results were identical for all but one individual. The most likely explanation remains the presence of a low-

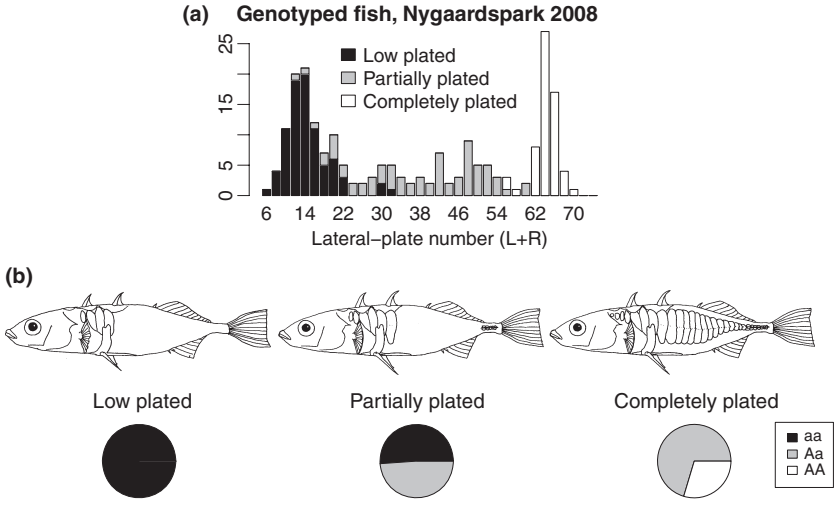

Fig. 1 Genetic architecture of the lateral-plate morphs. (a) The lateral plate number among the 319 fish of the 2008 population sample follows a trimodal distribution, justifying the classification into three phenotypic morphs (low, partial and completely plated). A few asymmetric fish appeared as misclassified when considering bilateral plate count because fish were classified according to one side only. (b) Around 50 individuals of each morph from the 2008 sample have been genotyped for polymorphisms associated with the *Eda* locus. The figure shows typical phenotypes for each morph, as well as their genotypic frequencies at the *Stn382* marker; allele *a* stands for the freshwater allele, and *A* for the ancestral, marine allele.

Table 2 Parameter estimates (95% confidence intervals) for the four models tested on the Nygaardspark pond data set. Lower AIC scores correspond to better models. Models involving frequency-dependent selection fit better, showing that the constant fitness models are too simple. Models assuming selection of genotypes outperform morph-based models in all situations

	Constant fitness	Frequency-dependent
Morph selection		
$\hat{p}_0$	0.336 (0.302–0.372)	0.145 (0.112–0.188)
$\hat{w}_L$	1.197 (1.154–1.241)	3.396 (2.657–4.339)
$\hat{w}_P$	1.137 (1.012–1.275)	1.248 (1.102–1.409)
$\hat{k}$		3.166 (2.460–3.938)
AIC	187	130
Genotype selection		
$\hat{p}_0$	0.103 (0.062–0.159)	0.112 (0.073–0.158)
$\hat{w}_{aa}$	1.920 (1.641–2.316)	2.736 (2.085–3.593)
$\hat{w}_{Aa}$	2.305 (1.884–2.895)	1.514 (1.116–2.091)
$\hat{k}$	—	1.269 (0.476–2.063)
AIC	124	116

frequency allele that cannot be visualized (no amplification, weak amplification or outcompeted alleles in the multiplex), making a number of heterozygotes *Aa* being mislabelled as *AA*. Consequently, subsequent analysis was carried out on *Stn382* only.

Genotyping confirms the almost complete monomorphism of the parental populations: the *a* allele, associated with the low-plated phenotype, has a frequency of 94% among the Eikelandsvatn fish, while the *A* 'marine' allele frequency in lake Myrdalsvatn is 100% (Tables S2 and S3, Supporting information). Among the 151 fish from the 2008 pond population, 75 were homozygote *aa*, 16 homozygote *AA* and 60 heterozygote. Fig. 1b and Table S4 (Supporting information) show the association between the *Eda* genotype and the phenotypic morph, and the link between genotype and plate number is illustrated in Fig. S1 (Supporting information).

Estimation of selection

The 'morph-selection model' relies on the assumption that the lateral-plate phenotype conditions the fitness of the fish. Assuming constant fitnesses, the fitness estimate of the partial morph appears to be very close to the complete morph ($\hat{w}_P = 1.14$, and $\hat{w}_L = 1.20$). All estimates and their associated confidence intervals are provided in Table 2, model fitting can be visualized in Fig. 2.

The 'genotype-selection model' assumes that fitness only depends on the genotype at *Eda* and thus that the changes in lateral armour phenotype reflect the effect of selection on the genotype at *Eda*, including hypothetical pleiotropic effects. The estimated fitness of the heterozygote is very high, $\hat{w}_{Aa} = 2.3$, and the fitness of the freshwater genotype, although lower, is not statistically significantly different ($\hat{w}_{aa} = 1.9$). The fit of the geno-

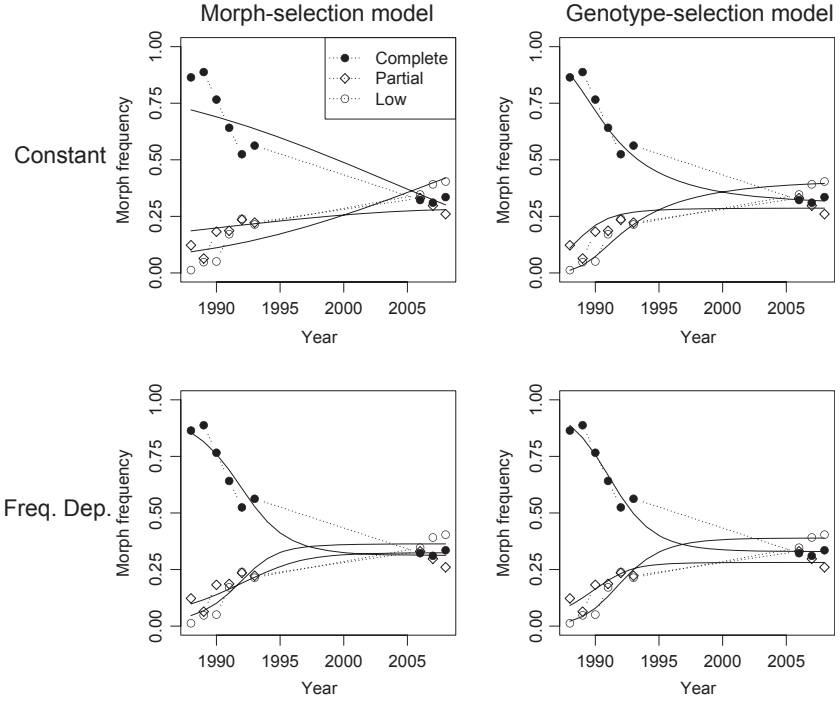

Fig. 2 The Nygaardspark pond time series, on which the two main models were fit (left: selection on morphs, right: selection on genotypes) for two cases: constant fitnesses (top), frequency-dependent selection (bottom). Models are described in the method section. Dots represent data points (black: completely plated, dark grey: partially plated, light grey: low plated), and lines illustrate the prediction from the maximum-likelihood parameter set.

type-selection model is better than the morph selection, which is reflected by a difference of 57 AIC units (Table 2).

The temporal pattern of the morph frequency, in particular the fact that morph frequencies seem to stabilize after the initial fast change, might be attributed to changes in the selection strenght. The possibility of frequency-dependent selection was thus tested. These models predict large fitness differences when the low morph is rare, and strong frequency-dependent selection: $\hat{k} = 1.3$ (the fitness of the *aa* genotype decreases by 12% when its frequency increases by 10%) to $\hat{k} = 3.2$ (the fitness of the low morph decreases by 27% when is frequency increases by 10%). Figure 3 illustrates the changes in relative fitnesses in the course of time based on this model.

Selection in a natural environment: Loberg Lake

Bell *et al.* (2004) reported an equivalent data set, with a 12-year time series from Loberg Lake (Alaska). Using our statistical framework on this data set and assuming a similar genetic architecture (slight model differences are described in the Methods section), fitness estimates for the full time series are $\hat{w}_L = 1.7$ and $\hat{w}_P = 1.5$ for the morph-selection model and rise to $\hat{w}_{aa} = 8.5$ and $\hat{w}_{Aa} = 8.3$ for the genotype-selection model (Fig. 4 and Table S5, Supporting information). The selection pattern thus appears to be similar in two unrelated situations: artificial introduction of polymorphic sticklebacks in a pond and natural invasion of marine sticklebacks in a lake. Yet, some differences could be observed: invasion was faster in Loberg lake, with stronger selection coefficients, and the pond dynamics seems to slow down at the end of the time series. Model selection provides the same conclusions, the genotype-selection model is favoured under the hypothesis of constant fitnesses, but there is strong evidence for a rapid decrease in the fitness advantage of the freshwater morph when its frequency increases ($\hat{k} = 1.9$). Genotype fitnesses are more stable, the gain is modest (three AIC units) when including frequency-dependent selection, and the frequency-dependence parameter is negative and close to 0 ($\hat{k} = -0.3$).

Discussion

Consistency of natural selection

Our results confirmed existing qualitative knowledge about stickleback adaptation and provided precise quantitative estimates. It is indeed already known that adaptation to freshwater environments is associated with a rapid loss of lateral plates; here, we show that

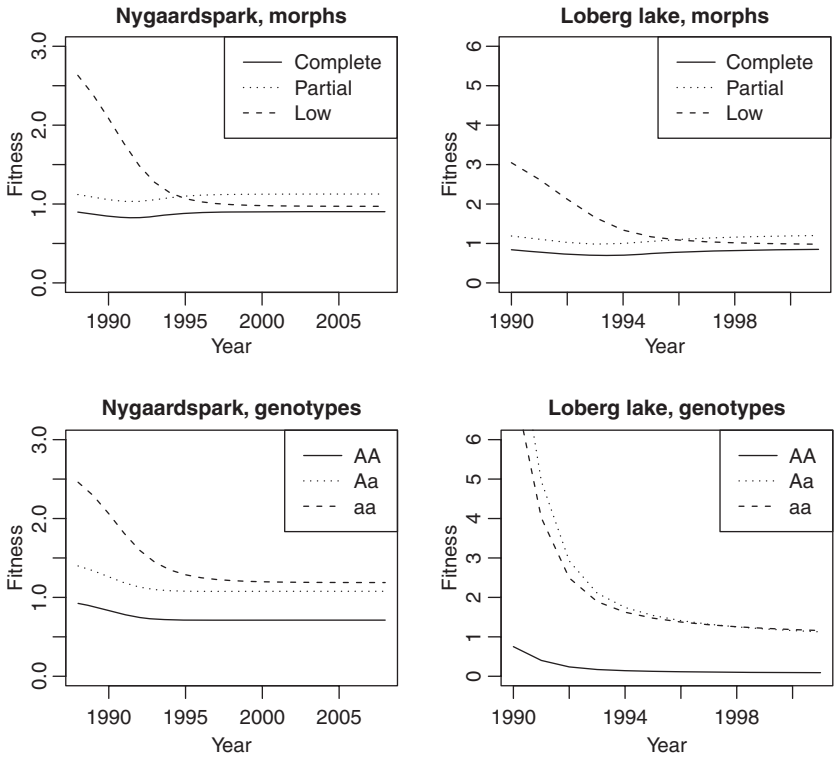

Fig. 3 Prediction of the fitness ($w/\bar{w}$) dynamics for the three morphs (top) and the three genotypes (bottom) based on the frequency-dependent selection model.

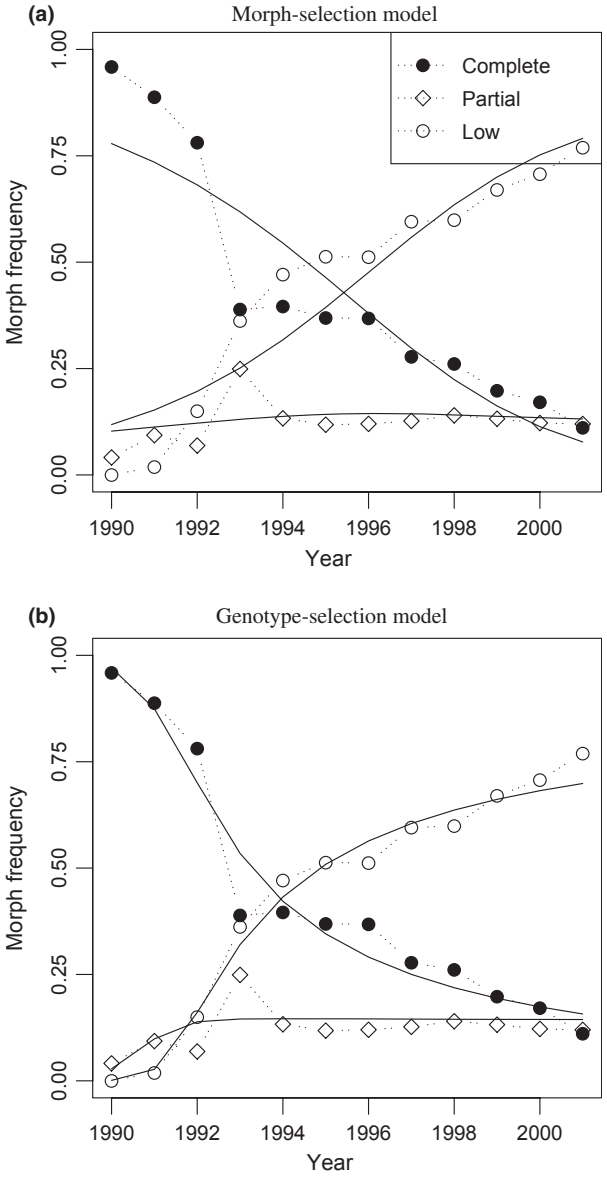

Fig. 4 Model fitting on the Loberg lake data from Bell *et al.* (2004). (a) constant selection on morphs, (b) constant selection on genotypes.

the fitness of the low-plated morph is at least 20% higher than the completely plated morph over the 20 years following the colonization of the experimental pond. This advantage raises to 92% when considering *aa* genotypes vs. *AA*.

The dynamics of the phenotypic change was quite smooth and regular, suggesting a consistent selection pressure in favour of the low-plated morph, and low effects of genetic drift and environmental stochasticity. The unique exception was the almost complete loss of low-plated fish at the very first generation, between 1987 and 1988. This observation remains striking and suggests an experimental accident rather than a selection event related to lateral plates. A small, nondestructive sample ($N = 20$) performed in spring 1988 showed 55% low-plated morphs, so low-plated fish survived the initial introduction and the first winter in the pond. The deficit of the low-plated morph in the fall of 1988 may thus be attributed to poor reproductive performance of these original low-plated fish. Incidentally, the initial rise in completely plated allele frequency, up to 90% according to our model-based estimates, increased the power of the experiment, because the time series came to cover a wider range of allelic frequencies, improving the accuracy of the statistical estimates of selection and retracing more precisely the evolution of a freshwater morph from a marine population.

The lateral armour of sticklebacks has been intensively studied, and additional material to estimate selection strength is available. The data provided by Bell *et al.* (2004) reanalysed above suggest that the selection pattern might be general, selection being even stronger in this natural lake. Interestingly, the loss of the complete morph slows down in both populations and even seems to halt in Nygaardspark. Some natural populations are known to remain polymorphic several centuries after the original colonization (Lucek *et al.* 2010), which supports the possibility that density dependence may lead to a stable polymorphic equilibrium, although longer experimental time series would be necessary to confirm this hypothesis.

Evolutionary models

The fitness estimates rely on population genetics models especially designed to correspond to the features of the experimental setting and the biological system. The advantage is twofold: (i) this procedure made it possible to estimate precisely parameters that would be otherwise difficult to measure and (ii) the analysis relies on a direct connection between theory and data. Maximum-likelihood methods provide confidence intervals, which describe the accuracy of parameter estimates, and base hypothesis comparison on solid statistical grounds, through model selection (Burnham & Anderson 2002).

Inaccuracies in the model may explain part of the residual variance, but are unlikely to strongly affect the estimates of selection strength. In particular, the real genetic architecture, which is known to involve additional small-effect genes (Aguirre *et al.* 2004; Colosimo *et al.* 2004), was simplified. The consequences of describing the phenotype based on three morphs are difficult to assess, and this simplifying assumption only works if the detailed features of the lateral armour do not matter for selection. Our intention here was to keep

models as simple as possible, while taking into account what is known to affect adaptation of sticklebacks from previous studies. Simulations (described in the Appendix S1, Supporting information) showed that the model is accurate and robust and that genetic drift or the sampling gap between 1994 and 2005 should not affect the main conclusions.

Three-parameter simple models (two constant fitnesses and the initial allele frequency) appeared to be outperformed by more complex models (frequency-dependent selection). However, identifying formally the underlying (nonexclusive) processes without additional experimental evidence seems difficult. Indeed, changes in fitness in the course of time can also be interpreted as environmental fluctuations (e.g. unstable ecosystem for several years after the initial introduction), demographic effects (e.g. carrying capacity achieved after a few years) or more complex evolutionary mechanisms, including e.g. linkage disequilibrium between *Eda* and a hypothetical linked locus under selection. Nevertheless, the smoothness of the evolutionary dynamics and the repeatability of the process support the hypothesis of a direct link between the invasion of freshwater-type fish and their associated decrease in fitness.

Selection and loss of body armour

The loss of the lateral armour plates is one of the most obvious phenotypic changes associated with the colonization of freshwater environments by marine sticklebacks, along with the loss of other armour structures, such as the pelvic spines, which genetic basis is also known (Chan *et al.* 2010). Several evolutionary hypotheses have been proposed to explain such a drastic phenotypic change, including e.g. differences in predation pressure (Hagen & Gilbertson 1973; Reimchen 1995, 2000; Bergstrom 2002; Vamosi & Schluter 2002; Marchinko 2009), or costs of developing calcified structures in freshwater (Giles 1983; Bell *et al.* 1993; Marchinko & Schluter 2007). Recently, Barrett *et al.* (2008) measured changes in allele frequencies for a single generation in artificial ponds. Their results suggest that the low-plated adults are indeed advantaged, but the completely plated genotype performs better at the juvenile stage. Overall, these effects were balanced (the low-plated allele loss at early stages is compensated by the adult fitness advantage) and thus do not explain the repeatable trend commonly observed in freshwater.

Our results show that selection is strong even when predation is limited, although predators may explain why selection intensity is higher in Loberg lake. Nevertheless, the question of the nature of selection mechanisms and the causes of the apparent frequency dependence (predation, diet, behaviour, sexual selection...) remains open and can probably not be resolved without further empirical work.

Interestingly, models attributing a fixed fitness to *Eda* genotypes, rather than plate phenotypes, appeared to fit experimental dynamics more convincingly than direct selection on lateral armour. Although the loss of the lateral armour plates is the most obvious phenotypic change associated with the colonization of freshwater environments by marine sticklebacks, it probably represents only a small part of the morphological, physiological and behavioural changes that are necessary to adapt to such a different life mode (Albert *et al.* 2008; Barrett 2010). Lateral-plate reduction might thus be not only becaused of fitness differences related to the presence or absence of calcified plates but also of indirect selection through pleiotropic effects of the *Eda* gene on one (or several) other traits under selection, as recently suggested by empirical evidence (Barrett *et al.* 2009).

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Data accessibility

Genotypic and phenotypic data deposited at Dryad: 10.5061/dryad.8412

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Results: statistical properties of the model.

Table S1 Morph definition.

Table S2 Parental populations (lakes sampled in 2008).

Table S3 Nygaardspark pond population (2008 sample).

Table S4 Conditional morph frequencies in the initial population

Table S5 Parameter estimates for the Loberg lake.

Fig. S1 Distribution of the lateral plate number according to the Eda genotype.

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