Open access • Journal Article • DOI:10.1111/J.1365-294X.2006.03190.X

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Published on: 29 Nov 2006 - Molecular Ecology (Wiley)

Topics: Population

Molecular Ecology (2007) **16**, 891–905 doi: 10.1111/j.1365-294X.2006.03190.x

Divergent selection as revealed by P_{ST} and QTL-based F_{ST} in three-spined stickleback (*Gasterosteus aculeatus*) populations along a coastal-inland gradient

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Abstract

Three measures of divergence, estimated at nine putatively neutral microsatellite markers, 14 quantitative traits, and seven quantitative trait loci (QTL) were compared in eight populations of the three-spined stickleback (*Gasterosteus aculeatus* L.) living in the Scheldt river basin (Belgium). Lowland estuarine and polder populations were polymorphic for the number of lateral plates, whereas upland freshwater populations were low-plated. The number of short gill rakers and the length of dorsal and pelvic spines gradually declined along a coastal-inland gradient. Plate number, short gill rakers and spine length showed moderate to strong signals of divergent selection between lowland and upland populations in comparison between P_{ST} (a phenotypic alternative for Q_{ST}) and neutral F_{ST}. However, such comparisons rely on the unrealistic assumption that phenotypic variance equals additive genetic variance, and that nonadditive genetic effects and environmental effects can be minimized. In order to verify this assumption and to confirm the phenotypic signals of divergence, we tested for divergent selection at the underlying QTL. For plate number, strong genetic evidence for divergent selection between lowland and upland populations was obtained based on an intron marker of the *Eda* gene, of which the genotype was highly congruent with plate morph. Genetic evidence for divergent selection on short gill rakers was limited to some population pairs where F_{ST} at only one of two QTL was detected as an outlier, although F_{ST} at both loci correlated significantly with P_{ST}. No genetic confirmation was obtained for divergent selection on dorsal spine length, as no outlier F_{ST}s were detected at dorsal spine QTL, and no significant correlations with P_{ST} were observed.

Keywords: adaptive divergence, phenotypic divergence, P_{ST}, Q_{ST}, QTL, quantitative traits

Received 3 July 2006; revision accepted 7 October 2006

Introduction

The ecology and phylogeography of the three-spined stickleback (*Gasterosteus aculeatus* L.) are ideal for interpreting the phenotypic consequences of genetic variation (Feder & Mitchell-Olds 2003). Recent developments in functional genomics have enhanced its role as a model vertebrate in ecology and evolution (Peichel *et al*. 2001; Colosimo *et al*. 2004, 2005; Cresko *et al*. 2004; Shapiro *et al*. 2004). In particular, the identification of genes underlying adaptive morphological traits has uncovered an important part of the molecular and genetic basis of parallel morphological evolution (Peichel *et al*. 2001; Schluter *et al*. 2004; Peichel 2005). Highly armoured, ancestral sticklebacks inhabit marine and estuarine habitats throughout the Northern Hemisphere. Multiple freshwater populations, characterized by reduced body armour, have evolved postglacially from them in a relatively short time frame of 10 000–16 000 years (Reusch *et al*. 2001; McKinnon *et al*. 2004; Raeymaekers *et al*. 2005). The rapid and parallel reduction of armour traits in freshwater populations has been linked to the presence of standing variation in ancestral marine populations for *Ectodysplasin* (*Eda*), a gene responsible for the number of lateral plates (Colosimo *et al*. 2005), and to variation in site-specific expression of the *Pitx1* gene, responsible for the development of the pelvic plate and spines (Shapiro *et al*. 2004).

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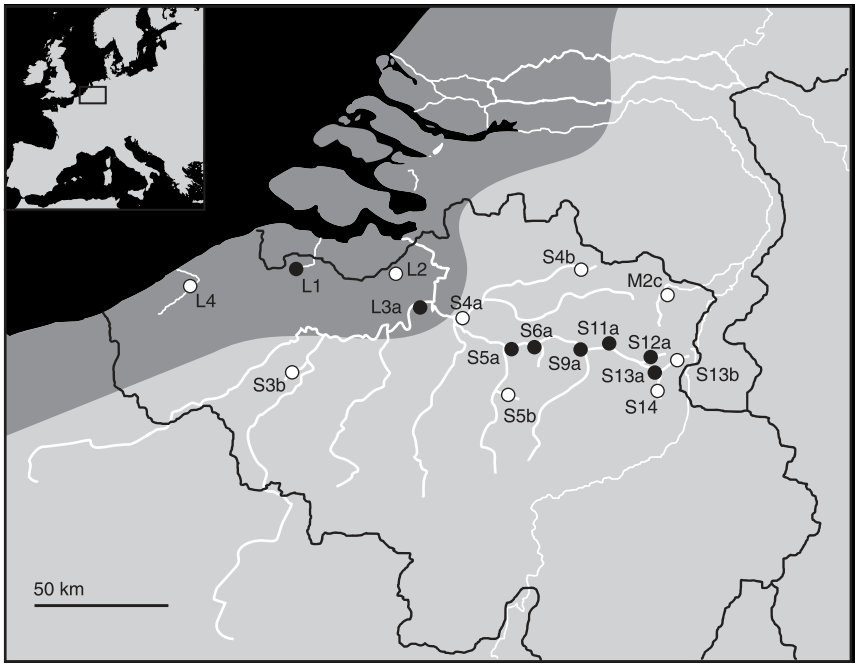

Fig. 1 Sampling locations of 17 populations of the three-spined stickleback (*Gasterosteus aculeatus*) in the North Sea polders, Scheldt river basin and Meuse river basin (Belgium). The shaded area refers to the distribution zone of lowland estuarine and polder populations. Black and white dots distinguish phenotyped from nonphenotyped samples, respectively. Population codes are indicated as in Table 1.

Further research to disentangle the molecular basis of phenotypic evolution is promising, given the diversity in morphologies, life histories and behaviours that sticklebacks from postglacial freshwater lakes and streams have developed in response to local ecological conditions (Peichel 2005). Interestingly, now that various morphological traits have been linked to quantitative trait loci (QTL), genetic variation at these traits, their underlying genes or candidate genes and putative neutral markers can be compared directly. Each type of genetic variation is likely to have its own geographical distribution (McKay & Latta 2002). Diversifying selection on quantitative traits should produce predictable patterns of allelic variation at the underlying QTL, or at loci that are very closely linked (Storz 2005). However, when gene flow is high and diversifying selection strong, adaptive divergence on polygenic traits may occur primarily as a result of covariance in allele frequencies among QTL, even in the absence of appreciable shifts in allele frequencies at single QTL (Latta 2003; Le Corre & Kremer 2003). Strong deviations from neutrality are not expected when large numbers of loci contribute to the variation in a divergently selected trait (Storz 2005).

Finding out which selective forces drive morphological evolution in sticklebacks remains difficult. For instance, many abiotic and ecological factors have been suggested to contribute to the evolution of body armour, including temperature (Bertin 1925), reduced levels of calcium (Giles 1983), salinity tolerance (Heuts 1947), swimming performance (Bergstrom 2002), as well as changes in predation regime (Hagen & Gilbertson 1973). None of these factors have been directly proven to account for the parallel evolution of the many freshwater populations across the Northern Hemisphere (Bell & Foster 1994). One problem is that it is difficult to estimate the adaptive value of different phenotypes with respect to some candidate selective forces. Polymorphism allowing for comparison within populations is often uncommon: most marine sticklebacks have a complete set of lateral plates, and most freshwater populations have only retained the anterior plates (Peichel 2005).

One region where populations polymorphic for plate number do occur is the downstream section of the West European great rivers (Meuse, Scheldt and Rhine) flowing into the southern bight of the North Sea (Heuts 1947; Wootton 1976; Raeymaekers *et al.* 2005; Fig. 1). These 'lowland' populations are anadromous or landlocked, and live in estuaries or polder creeks. The latter represent dyked, seminatural brackish and freshwater habitats of Holocene origin with varying connectivity to adjacent estuaries or the open sea. Lowland populations contain varying percentages of unkeeled low-plated, keeled low-plated, keeled partially plated and keeled completely plated sticklebacks. Locally, the various morphs may occur throughout the year (Raeymaekers, unpublished), indicating true polymorphism rather than a seasonal mix of marine and freshwater populations. Observations by Heuts (1947), recently confirmed by Raeymaekers *et al.* (2005), indicate that lowland plate morph variation reflects true polymorphism and neither immigration of low-plated upland freshwater sticklebacks nor hybridization. The plate polymorphism is remarkable because in most coastal areas, the three plate morphs usually only mix when low-plated freshwater populations and completely plated anadromous populations

hybridize, resulting in a low frequency of partially plated fish (e.g. see Jones *et al*. 2006). The West European divergence between lowland and upland populations represents a specific case of the anadromous-freshwater system (McKinnon & Rundle 2002). Most important, comparisons among plate morphs in the lowlands may reveal what is favouring the low-plated morph in upland freshwater habitats. The geographical range of the lowland–upland system extends from the southern North Sea to the western Baltic (Münzing 1963; Wootton 1976). These populations clustered with low bootstrap support in a microsatellite-based phylogeny (Mäkinen *et al*. 2006). The area has been interpreted as marking the secondary contact zone between marine and freshwater sticklebacks following the retreat of the Late Pleistocene ice sheet (Münzing 1963; Wootton 1976). However, there are no indications for Pleistocene refugia of freshwater sticklebacks farther north than in the Mediterranean region (Mäkinen *et al*. 2006).

We analyse here the divergence between lowland and upland populations, acknowledging that three-spined sticklebacks represent a unique opportunity to test in the wild for the influence of divergent selection on both quantitative traits and their underlying genes. We calculated morphological divergence between natural populations as P_{ST}, a phenotypic alternative for Q_{ST} (Spitze 1993) which estimates genetic divergence at quantitative traits. Divergence in quantitative traits should be similar to divergence in allele frequencies at nuclear loci, if they are evolving neutrally and have an additive genetic basis (Wright 1951). Under the influence of migration, mutation and genetic drift, the among-population proportion of total genetic variance in phenotypic traits is expected to equal that of neutral molecular loci (Lande 1992). A method for detection of natural selection involves the comparison of the level of differentiation at quantitative traits with the differentiation at neutral molecular markers (F_{ST}). The prediction is that divergent selection will cause the divergence in quantitative traits to be larger than expected from neutral loci (Merilä & Crnokrak 2001; Bernatchez 2004). Although P_{ST} has been interpreted as Q_{ST} (Merilä 1997; Storz 2002; Bernatchez 2004; Ostbye *et al*. 2005; but see Leinonen *et al*. 2006), we prefer to distinguish between both indices because P_{ST} is based on natural populations. P_{ST} can only be compared with F_{ST} under the assumption that phenotypic variance equals additive genetic variance, excluding nonadditive genetic and environmental effects (Merilä 1997). As this assumption is usually unrealistic, we did not only compare P_{ST} with genetic divergence at putatively neutral microsatellite loci, but also with genetic divergence at quantitative trait loci (QTL) known to affect the phenotypic traits studied. These loci were selected from the QTL maps of the two most studied stickleback 'species pairs' (McKinnon & Rundle 2002). The first study (Peichel *et al*. 2001) mapped several important morphological traits in the regional benthic-limnetic system: short gill rakers, first and second dorsal spine, pelvic spine, and lateral plates. The second study (Colosimo *et al*. 2005) identified the *Eda* gene in the worldwide anadromous-freshwater system.

Characterizing the lowland–upland system along a coastal–inland gradient, we first look for traits that show indirect signals of divergent selection as revealed by P_{ST}. Second, we exploit the information available from the linkage maps developed by Peichel *et al*. (2001) and Colosimo *et al*. (2005). To evaluate whether the phenotypic signals of divergent selection can be confirmed genetically, we test whether the QTL underlying the divergent quantitative traits can be detected as outlier loci. We also investigate whether P_{ST} and the corresponding QTL-based F_{ST}s are correlated. We discuss the likelihood to observe relationships in natural populations between phenotypic traits under divergent natural selection and loci derived from QTL maps, of which one is most relevant for the young and regional benthic-limnetic system (Peichel *et al*. 2001), and another for the ancient and widely distributed anadromous-freshwater system (Colosimo *et al*. 2005).

Materials and methods

Sample collection

Three-spined sticklebacks (*Gasterosteus aculeatus*) were sampled from four lowland and 13 upland locations in Belgium in spring 2002 (Fig. 1; Table 1). The lowland populations inhabited the polders bordering the Scheldt estuary (L1, L2, L3a) and the North Sea (L4). Eight upland populations (S4a, S5a, S6a, S9a, S11a, S12a, S13a, S14) originated from near the main channel of the Scheldt river basin (tracing the rivers Scheldt, Rupel, Nete, Dijle and Demer). Four upland populations (S3b, S4b, S5b, S13b) were from small upstream tributaries of the Scheldt basin, and one upland population (M2c) was from the Belgian part of the Meuse river basin. Fifty adult individuals per site were caught with a dip net or by electrofishing, and flash frozen on dry ice. All individuals were weighed (±0.01 g) and measured for standard length (SL) and total length (TL) (±0.1 cm). Fin clips were taken and stored in 100% ethanol for DNA analysis.

All samples were genotyped at six neutral microsatellite loci (i.e. multiplex 1; see below) to reconstruct the overall phylogenetic relationships. Focus of this study was on a subset of eight populations which were selected along a coastal–inland gradient, including lowland samples L1 and L3a, and upland samples S5a, S6a, S9a, S11a, S12a and S13a. These samples were genotyped at three additional neutral microsatellite loci (hence nine in total) and seven QTL (Table 2). A subset of 30 individuals of each of these samples was phenotyped in detail (see below).

Table 1 Characteristics of 17 sampling locations of three-spined stickleback populations in Belgium, genotyped at six microsatellite loci. H_E, expected (unbiased) heterozygosity; H_O, observed heterozygosity; AR, allelic richness calculated for 18 individuals. (L), lowland population; (U), upland population

Population	Code	Basin	H_E	H_O	AR	Latitude	Longitude
Westkerke (L)	L4	North Sea	0.87	0.81	11.46	51°10.000″	3°00.000″
St Laureins (L)	L1	Scheldt estuary	0.8	0.78	9.55	51°14.680″	3°32.038″
St Gillis Waas (L)	L2	Scheldt estuary	0.83	0.82	10.13	51°13.582″	4°06.669″
Niel (L)	L3a	Scheldt estuary	0.87	0.88	11.23	51°06.883″	4°14.170″
Lede (U)	S3b	Scheldt	0.76	0.77	7.27	50°53.200″	3°34.390″
Mechelen (U)	S4a	Nete	0.83	0.83	10.34	51°03.734″	4°27.588″
Dessel (U)	S4b	Nete	0.7	0.66	7.59	51°13.933″	5°06.969″
Werchter (U)	S5a	Demer	0.78	0.77	9.46	50°57.806″	4°43.276″
Vaalbeek (U)	S5b	Dijle	0.65	0.63	6.22	50°49.466″	4°40.105″
Aarschot (U)	S6a	Demer	0.79	0.76	8.20	50°58.831″	4°50.898″
Zelem (U)	S9a	Demer	0.73	0.72	7.66	50°57.761″	5°05.431″
Kermt (U)	S11a	Demer	0.73	0.72	7.56	50°57.900″	5°14.043″
Diepenbeek (U)	S12a	Demer	0.79	0.73	7.59	50°55.409″	5°27.509″
Bilzen (U)	S13a	Demer	0.81	0.83	7.73	50°53.749″	5°29.290″
Zutendaal (U)	S13b	Demer	0.64	0.66	4.37	50°54.539″	5°34.006″
Alt-Hoeselt (U)	S14	Demer	0.79	0.71	7.39	50°50.479″	5°30.031″
Bree (U)	M2c	Maas	0.71	0.57	5.86	51°08.408″	5°34.119″

Table 2 Pearson correlations (R) between pairwise P_{ST} at single phenotypic traits and (a) pairwise F_{ST} at nine putative neutral microsatellite loci, and (b) pairwise F_{ST} at single quantitative trait loci (QTL), calculated among eight three-spined stickleback populations. Partial correlations for QTL control for F_{ST} at neutral loci. LG, linkage group according to Peichel *et al*. (2001), Colosimo *et al*. (2004) or Colosimo *et al*. (2005). Stn381 is linked to the *Eda* gene. The five correlations in bold are plotted in Fig. 6

	(a)	(b) QTL			
Trait and code	Neutral loci Simple R	Locus	QTL (LG)	Simple R	Partial R
1st dorsal spine length (DS-1)	0.14	Stn9	*Spine1-a* (I)	**0.34**	0.31
2nd dorsal spine length (DS-2)	0.07	Stn96	*Spine2-a* (VIII)	0.10	0.08
		Stn130	*Spine2-b* (XI)	0.11	0.08
		Stn131	*Spine2-b* (XI)	−0.14	−0.16
Number of frontal short gill rakers (SGR-1)	0.07	Stn130	*Raker#-a* (XI)	**0.50***	0.52*
		Stn131	*Raker#-a* (XI)	0.43	0.43
		Stn178	*Raker#-b* (XVI)	−0.32	−0.36
Number of distal short gill rakers (SGR-2)	**0.52***	Stn130	*Raker#-a* (XI)	0.33	0.13
		Stn131	*Raker#-a* (XI)	0.01	−0.17
		Stn178	*Raker#-b* (XVI)	**0.56***	0.48*
Number of anterior nonstructural plates (P1P3)	0.05	Stn381	*Eda* (IV)	0.88**	0.88**
Number of central structural plates (P4P8)	0.07	Stn381	*Eda* (IV)	0.97**	0.97**
Number of posterior nonstructural plates (P9PN)	−0.02	Stn381	*Eda* (IV)	0.96*	0.96*
Total number of plates (P1PN)	0.02	Stn381	*Eda* (IV)	**0.92***	0.92*
Plate size (–)	NA	Gac1125	–(XXV)	NA	NA

*$P < 0.05$; **$P < 0.01$; P values obtained from 10 000 simple or partial Mantel test permutations.

Morphometrics

Pectoral fin rays (PFR) were counted bilaterally. Fish bodies were fixed in a 4% formalin solution. After 2 months, the sticklebacks were rinsed with water for 72 h, bleached for 4 h (1% KOH bleach solution), buffered for 24 h (30% borax buffer, pH 7.0) and stained with alizarin red S to facilitate plate counts and plate morph registration (Taylor & Van Dyke 1985). After staining, we determined body depth (coded BD) and the diameter of both eyes (coded EYE) with a digital calliper (±0.01 mm). BD was measured connecting the highest point (i.e. between the first and second spine) and the lowest point of the fish (i.e. on the pelvic plate), perpendicular to the lateral line. The eye

diameter was measured along a vertical straight line on the bony structure of the eye. Both pelvic spines (PS) and the first (DS-1) and second (DS-2) dorsal spine were removed from the fish and stored separately in 100% glycerol. Spines were photographed with a Matrox Meteor camera connected with the software program DAS (Cam2Disk 2.2) and digitally measured in Image-Pro Plus 5.0. Spine length was determined as the straight line connecting the lowest point of the articulation to the spine tip (Mazzi *et al*. 2002). The number and position of the lateral plates on the right and left sides were determined. The distinction was made between anterior nonstructural plates (P1P3), central structural plates (P4P8) and posterior nonstructural plates (P9PN) (Bergstrom & Reimchen 2003). Total plate number (P1PN) was calculated as the sum of P1P3, P4P8 and P9PN. Based on this number, specimens were categorized as low-plated (10 or fewer plates), partially plated (11–20 plates), or completely plated (21–30 plates). The presence of a keel, a small modification of the caudal lateral plates, was noted. Gills were dissected after removal of the gill cover. Large (LGR) and small (SGR) gill rakers were counted on the frontal (LGR-1; SGR-1) and distal (LGR-2; SGR-2) part of the first branchial arch (Peichel *et al*. 2001). The whole procedure was repeated; final values for all traits were obtained after averaging over two independent registrations. In the case of bilateral traits, values were also averaged over left and right.

DNA extraction and genotyping

Genomic DNA was extracted from fin clips using a silica-based purification method (Elphinstone *et al*. 2003). The amplification of loci was organized in three multiplex reactions. The first multiplex reaction contained six loci (Gac5196, Gac2111, Gac4170, Gac1097, Gac7033, Gac1125) from Largiadèr *et al*. (1999). The second multiplex reaction contained four loci (Stn9, Stn96, Stn131, Stn174) from Peichel *et al*. (2001) and one locus (Stn381) from Colosimo *et al*. (2005). The third multiplex reaction contained five loci (Stn23, Stn37, Stn84, Stn130, Stn131) from Peichel *et al*. (2001). Loci were amplified with the QIAGEN Multiplex PCR Kit (QIAGEN). The 12.5 μL reactions contained 1–100 ng genomic DNA, forward and reverse primer, 1 × QIAGEN Multiplex PCR mastermix (3 mM $MgCl_2$) and RNase-free water. Final reaction volume primer concentrations were 0.05 μM (Gac1125, Gac5196, Gac2111, Gac7033), 0.10 μM (Stn84), 0.15 μM (Stn96, Stn174, Stn381), 0.20 μM (Stn23, Stn37, Stn131, Stn178), 0.30 μM (Stn130), 0.75 μM (Gac4170), 0.80 μM (Stn9) or 1 μM (Gac1097). Reactions consisted of an initial activation step of 15 min at 95 °C, followed by 30 cycles (multiplex 1) or 35 cycles (multiplex 2 and 3) of 30 s at 94 °C, 90 s at 56 °C (multiplex 1) or 90 s at 55 °C (multiplex 2 and 3), and 1 min at 72 °C. A final elongation step of 10 min at 72 °C was performed. Polymerase chain reaction products were visualized on an ABI3130 Avant Genetic analyser (Applied Biosystems). Allele sizes were determined by means of an internal GENESCAN 500-LIZ size standard and genotypes were scored using GENEMAPPER 3.7 (Applied Biosystems).

Data analysis

Genetic diversity at neutral loci and QTL was evaluated based on genotype and allele frequencies, the level of polymorphism, and the observed and unbiased expected heterozygosity (H_O and H_E) using GENETIX 4.04 (Belkhir *et al*. 2002). Allelic richness (AR) was quantified in FSTAT 2.9.3.2 (Goudet 1995) and averaged over loci. Population differentiation was quantified for each locus in GENETIX using the standardized allelic variance F_{ST}, estimated as θ (Weir & Cockerham 1984). Pairwise F_{ST} values at neutral loci were visualized by a two-dimensional nonmetric multidimensional scaling (NMDS) plot with the function ISOMDS in S-PLUS (MathSoft).

Phenotypic differences among samples were quantified for meristic traits (gill rakers, lateral plates and pectoral fin rays) using one-way analysis of variance (ANOVA) and post-hoc Tukey tests. Morphometric traits, correlated with body length (eye diameter, body depth, pelvic spine length, first and second dorsal spine length) were allometrically scaled prior to comparison. Common within-group regression slopes between each $\log_{10}$-transformed morphometric trait and $\log_{10}$-transformed standard body length were estimated using ANCOVAS without interaction. Slopes were used to correct each fish to the overall mean standard body length (4.96 cm) as incorporated into the equation (see Hendry *et al*. 2002):

$$M_{STD} = M_O(4.96/SL_O)^b$$

where M is the trait size, SL is standard body length, b is the ANCOVA slope with the interaction term removed, and subscripts STD and O refer to standardized and observed measurements. Standardized trait sizes were compared among samples using one-way ANOVAS and Tukey tests.

In order to detect indirect (phenotypic) signals of divergent selection, we compared the extent of divergence for meristic and standardized morphometric traits, quantified as P_{ST}, with neutral molecular divergence (F_{ST}). Under divergent selection, P_{ST} will be larger than expected on the basis of neutral loci (Bernatchez 2004; Leinonen *et al*. 2006). Because of the potential inflation of phenotypic divergence by nonadditive genetic effects and environmental effects, we compared P_{ST} with genetic divergence at the underlying QTL. If a trait is under divergent selection, not only P_{ST} but also F_{ST} at QTL should be larger than expected on the basis of neutral loci (Vasemägi & Primmer 2005). The outcome for F_{ST} at QTL should depend on the number of QTL underlying the respective trait; a large number of loci

complicate the detection of deviations from neutrality at the individual QTL (Latta 2003). We also test over the entire set of population pairs whether P_{ST} at a phenotypic trait and F_{ST} at the corresponding QTL are correlated.

Equivalent to Q_{ST} (Spitze 1993), which quantifies the proportion of among-population genetic variance in quantitative traits, P_{ST} quantifies the proportion of among-population phenotypic variance in quantitative traits:

$$P_{ST} = \sigma^2_{GB}/(\sigma^2_{GB} + 2\sigma^2_{GW})$$

where σ^2_{GB} and σ^2_{GW} are the among-population and within-population variance components for a phenotypic trait in the wild, respectively. Phenotypic variance components were estimated following Sokal & Rohlf 1995 (pp. 208–217), using the type I method implemented in a PROC VARCOMP macro in SAS 9.1. P_{ST} at each trait was estimated between all population pairs.

To compare the level of phenotypic divergence with neutral genetic divergence, pairwise P_{ST} values for single traits were compared with pairwise F_{ST}s calculated at nine neutral loci. Similarly, to compare genetic divergence at single QTL with neutral genetic divergence, pairwise F_{ST}s at single QTL were compared with pairwise neutral F_{ST}s. Significance was assessed with 95% confidence intervals for lowland–lowland (one combination), lowland–upland (12 combinations) and upland–upland (15 combinations) population pairs separately. To test whether levels of phenotypic divergence, genetic divergence at single QTL and neutral genetic divergence are correlated, we calculated and tested the correlations between pairwise P_{ST}, QTL-based F_{ST} and neutral F_{ST} (n = all 28 population pairs) with a simple Mantel test module (Mantel 1967) programmed in S-PLUS. For reasons explained in the Discussion, we also tested correlations between pairwise P_{ST} and QTL-based F_{ST} after control for neutral F_{ST}, using partial Mantel tests.

To identify selective footprints at QTL in single population pairs, we used a coalescence-based simulation approach (the *F*-test), developed by Vitalis *et al*. (2001). This method relies upon a population-split model from the common ancestor population and uses the population-specific parameters of population divergence, *F*, conditional on the number of alleles. The expected joint distributions of F_{pop1} and F_{pop2} were generated by performing 100 000–500 000 coalescent simulations for each population pair using the software DETSEL 1.0 (Vitalis *et al*. 2003). The following nuisance parameters were used in different combinations to generate null distributions with a similar number of allelic states as in the observed data set: mutation rate (μ) (infinite allele model — IAM) 0.001 and 0.0001; ancestral population size (N_e) 1000 and 10 000; population size before the split (N_O) 50; time since an assumed bottleneck event (T_O) 50 and 1000 generations; time since the population split (t) 50 and 1000 generations. Loci that consistently fall outside the specified 95%'probability region' in comparison with the simulated data points are reported as potentially being affected by selection.

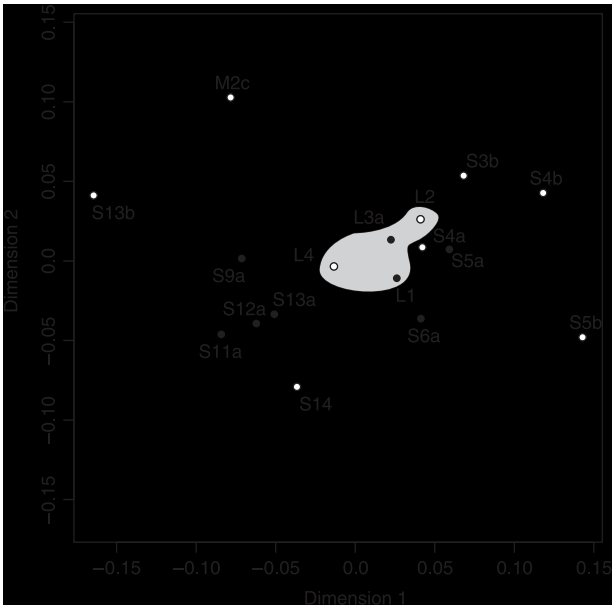

Fig. 2 Nonmetric multidimensional scaling (NMDS) plot based on pairwise F_{ST} values among 17 three-spined stickleback populations calculated from the genotypes at six microsatellite loci. The shaded area groups populations of the lowland ecotype. Black and white dots distinguish phenotyped and nonphenotyped samples, respectively. Population codes are indicated as in Table 1.

Results

Genetic diversity and population structure at neutral loci

The four lowland populations were characterized by a higher allelic richness (AR) compared to the 13 upland populations (lowland, AR = 10.59; upland, AR = 7.48; P = 0.0045; Table 1). Lowland populations did not display a significantly lower genetic differentiation than upland populations (lowland, F_{ST} = 0.056; upland, F_{ST} = 0.157; P = 0.096). Nevertheless, they clustered in a central position in a pairwise F_{ST}–based NMDS plot (Fig. 2). Two upland populations from the lowest downstream positions in the Scheldt basin (S4a and S5a) were characterized by a higher AR and closer affinities to lowland populations than other upland populations. AR did not decline further upstream up to some small upstream rivers (S3b, S4b, S5b and S13b; Table 1; Fig. 2). Levels of gene flow suggest that our eight focal populations (Fig. 2; black bullets) are not genetically isolated.

Phenotypic differences

All phenotypic traits, except for the number of distal long gill rakers, differed among populations (Fig. 3). Armour

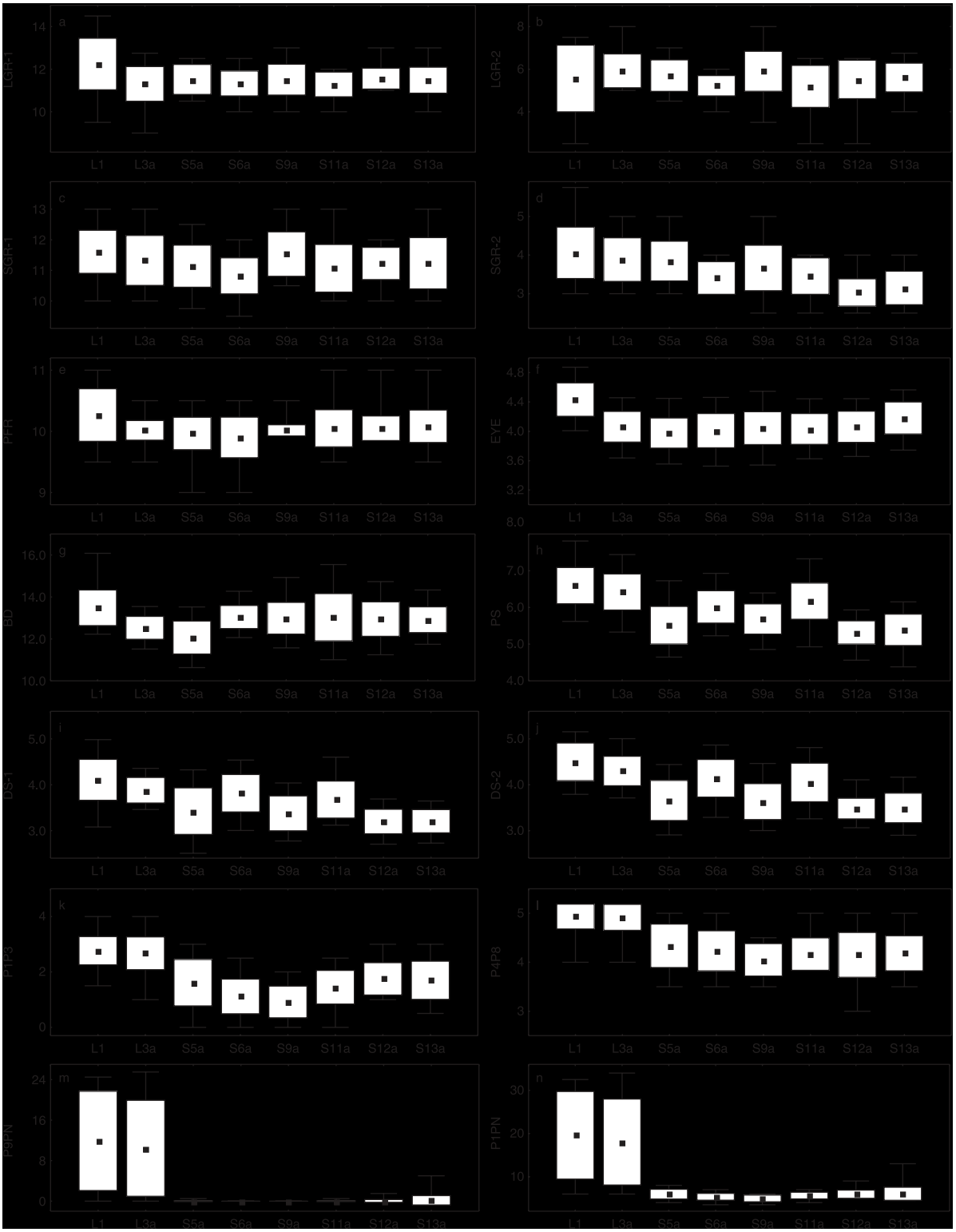

Fig. 3 Divergence at 14 phenotypic traits among eight three-spined stickleback populations from the Scheldt basin in Belgium. (a) Number of frontal long gill rakers; (b) number of distal long gill rakers; (c) number of frontal short gill rakers; (d) number of distal short gill rakers; (e) number of pectoral fin rays; (f) standardized eye diameter (mm); (g) standardized body depth (mm); (h) standardized length of pelvic spines (mm); (i) standardized length of first dorsal spine (mm); (j) standardized length of second dorsal spine (mm); (k) number of anterior nonstructural plates; (l) number of central structural plates; (m) number of posterior nonstructural plates; (n) total number of lateral plates. Box and tails indicate standard deviation and minimum–maximum, respectively. Population codes are indicated as in Table 1.

Table 3 Contingency of the genotype at locus Stn381 with lateral plate morph in eight three-spined stickleback populations. (L), lowland population; (U), upland population. LNK, low-plated, unkeeled; LK, low-plated, keeled; PK, partially plated, keeled; CK, completely plated, keeled. Three LNK individuals with rare genotypes originating from three populations were excluded from this table (S5a: 191–193; S12a: 167–193; S13a: 185–185). An individual from L3a (not phenotyped) had an additional rare genotype (175–187). Population codes are as in Table 1

Population	Stn381	LNK	LK	PK	CK	Population	Stn381	LNK	LK	PK	CK
L1 (L)	175–175				8	S9a (U)	175–175				
	175–193	1	5	8	5		175–193	2			
	193–193	1	2				193–193	27			
L3a (L)	175–175	1		2	5	S11a (U)	175–175				
	175–193	1	2	8	4		175–193				
	193–193	4					193–193	30			
S5a (U)	175–175					S12a (U)	175–175	1			
	175–193	5					175–193	2			
	193–193	19					193–193	24			
S6a (U)	175–175					S13a (U)	175–175				
	175–193						175–193				
	193–193	30					193–193	27		1	

traits were typically more pronounced in lowland populations (L1 and L3a). Each of the three types of lateral plates (P1P3, P4P8 and P9PN) contributed to an overall higher plate number in these populations. In terms of plate morph variation, both populations were inhabited by about equal ratios of low-plated, partially plated and completely plated individuals (Table 3). Posterior nonstructural plates were absent in upland populations, except in one individual in S13a. The number of anterior nonstructural plates was significantly lower in S6a and S9a compared to other upland populations. The length of the first and second dorsal spine, and pelvic spines gradually decreased upstream. There was also such a decline in the number of distal short gill rakers, with the lowest values measured in S12a and S13a. Other traits merely characterized single populations. S6a had a low number of frontal short gill rakers, and S5a had a small body depth. Population L1 showed a high number of frontal long gill rakers, a high number of pectoral fin rays, and a large eye diameter. Therefore, population L3a — situated at the edge of the polder area — was more upland-like than population L1.

Phenotypic signals of divergent selection

P_{ST} values at armour traits in lowland–upland population pairs (plate numbers and all spine lengths) were significantly higher than neutral F_{ST} (Fig. 4a). P_{ST}s for plate number but not for spine length in lowland–upland population pairs significantly exceeded P_{ST}s in upland population pairs. P_{ST} values at other traits (number of long and short gill rakers, eye diameter, body depth, number of fin rays) did not consistently differ with respect to the lowland–upland categorization of population pairs, and were not higher than neutral F_{ST}. P_{ST} for the single lowland population pair was small for distal short gill rakers, spines, and lateral plates (pointing to uniform selection), but large for frontal long gill rakers, eye diameter and body depth.

Genetic signals of divergent selection

Expected heterozygosities (H_E) were in general higher in grouped lowland populations than in grouped upland populations (Fig. 5). H_E at locus Stn381, a diagnostic indel linked to the *Eda* gene, was low in lowland populations, and extremely low in upland populations. Such discrepancy may indicate genetic hitchhiking as an effect of positive selection (Kaplan *et al.* 1989; Storz 2005). Stn381 was present with two common alleles (175 bp and 193 bp; Table 3). Genotype and plate morphology matched in most cases: homozygotes for the small common allele were completely plated and keeled, whereas homozygotes for the large common allele were low-plated (keeled and unkeeled). Heterozygous individuals were low-plated, partially plated or completely plated. Consequently, and consistent with the result on P_{ST} at plate number (Fig. 4a), lowland–upland pairwise F_{ST}s largely exceeded both neutral F_{ST}s and upland pairwise F_{ST}s (Fig. 4b). Upland pairwise F_{ST} at Stn381 was significantly lower than neutral F_{ST}. Upland pairwise P_{ST} at central structural plates and at posterior nonstructural plates was also significantly lower than neutral F_{ST}, in contrast to the anterior nonstructural plates (Fig. 4a). It indicates that the same specific subtraits are the target of selection favouring the low-plated phenotype in different upland populations (Merilä & Crnokrak 2001).

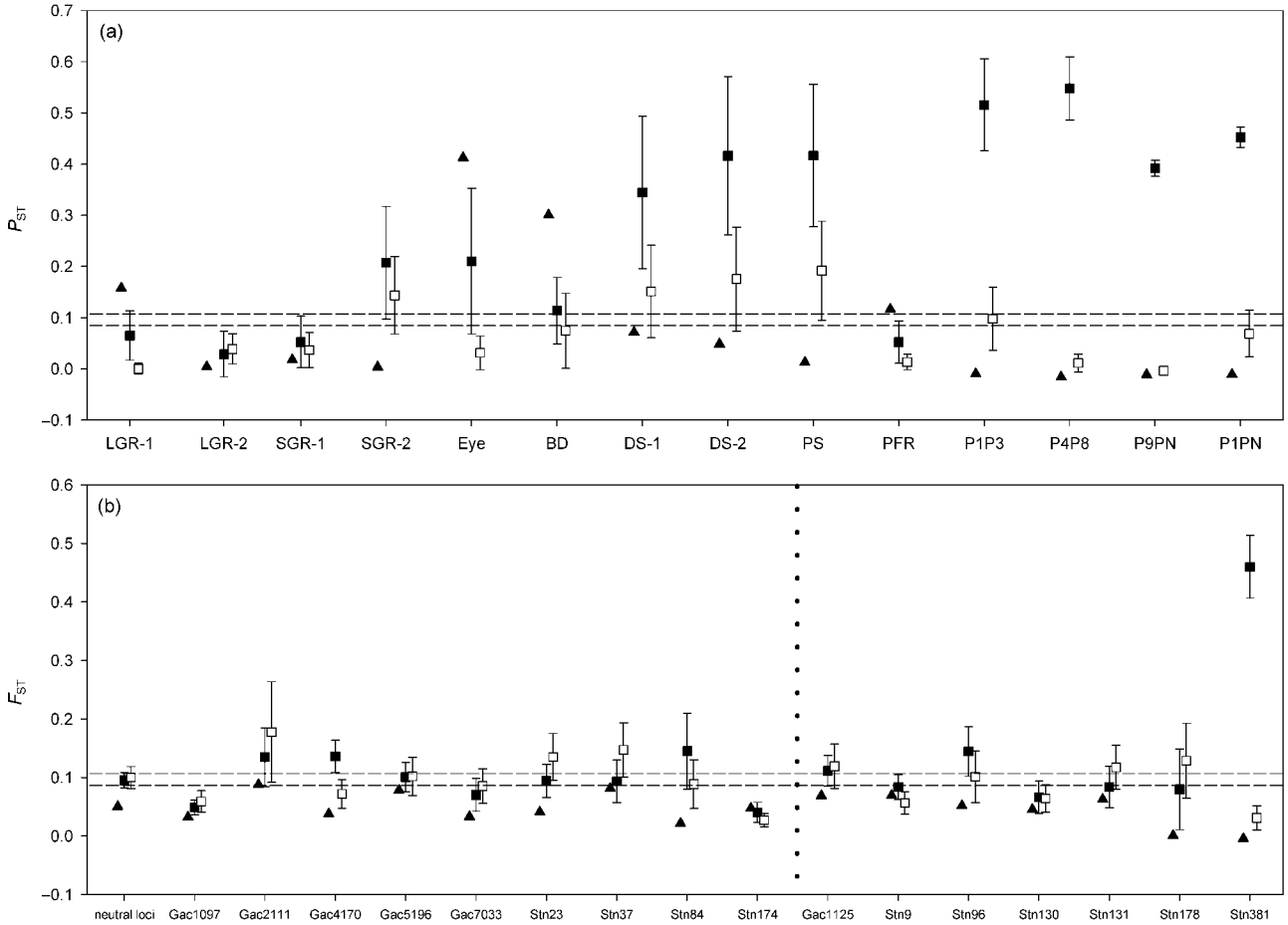

Fig. 4 Pairwise P_{ST} at 14 traits (a) and pairwise F_{ST} at 16 loci (b) among eight three-spined stickleback populations from the Scheldt basin in Belgium. Triangles, black squares and white squares denote lowland (1 comparison), lowland–upland (12 comparisons) and upland population pairs (15 comparisons), respectively. Tails denote 95% confidence intervals and horizontal dashed lines delineate 95% confidence interval for F_{ST} as estimated from nine neutral loci. (a) LGR-1, number of frontal long gill rakers; LGR-2, number of distal long gill rakers; SGR-1, number of frontal short gill rakers; SGR-2, number of distal short gill rakers; EYE, eye diameter; BD, body depth; DS-1, length of first dorsal spine; DS-2, length of second dorsal spine; PS, length of pelvic spines; PFR, number of pectoral fin rays; P1P3, number of anterior nonstructural plates; P4P8, number of central structural plates; P9PN, number of posterior nonstructural plates; P1PN, total number of lateral plates. (b) The vertical line divides nine putative neutral loci (left) and seven QTL (right).

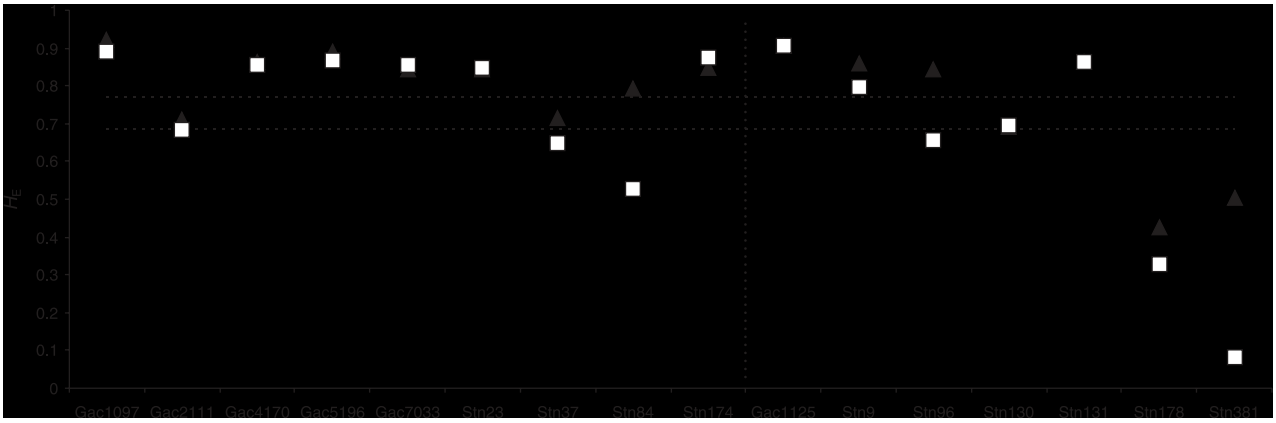

Fig. 5 Expected heterozygosity (H_E) at nine putative neutral loci (left) and seven QTL (right) for grouped lowland (triangles) and upland (squares) three-spined stickleback populations from the Scheldt basin. The upper and lower horizontal dashed lines delineate *lower* 95% confidence limits for neutral H_E in lowland and upland populations, respectively.

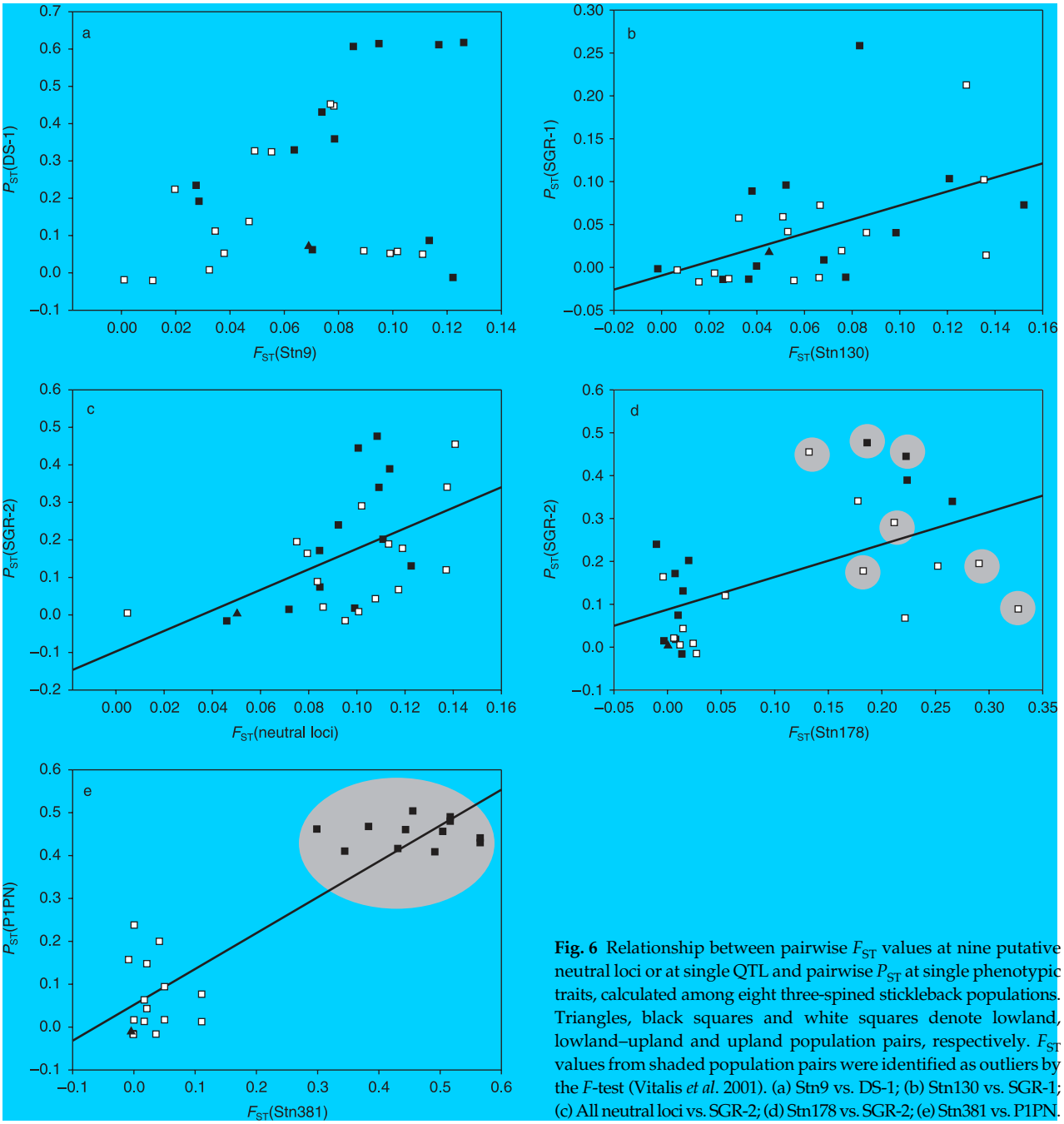

Fig. 6 Relationship between pairwise F_{ST} values at nine putative neutral loci or at single QTL and pairwise P_{ST} at single phenotypic traits, calculated among eight three-spined stickleback populations. Triangles, black squares and white squares denote lowland, lowland–upland and upland population pairs, respectively. F_{ST} values from shaded population pairs were identified as outliers by the *F*-test (Vitalis *et al.* 2001). (a) Stn9 vs. DS-1; (b) Stn130 vs. SGR-1; (c) All neutral loci vs. SGR-2; (d) Stn178 vs. SGR-2; (e) Stn381 vs. P1PN.

Lowland–upland categorization of F_{ST}s at locus Stn96 (linked to *Spine2-a*) revealed a pattern similar to P_{ST} at the second dorsal spine (Fig. 4a, b). However, F_{ST}s were smaller than P_{ST}s and in the same range as neutral F_{ST}. Other QTL for the second dorsal spine (Stn130 and Stn131: *Spine2-b*), the first dorsal spine (Stn9: *Spine1-a*) and the number of short gill rakers (Stn130 and Stn131: *Raker#-a*; Stn178: *Raker#-b*) seemed of low importance regarding the lowland–upland divergence, and the corresponding F_{ST} values did not exceed neutral F_{ST} (Fig. 4b).

P_{ST}-F_{ST} *correlations*

Among the 14 correlations over the entire set of population pairs between pairwise P_{ST} and pairwise F_{ST} at single QTL, six were significantly positive (Table 2): the number of frontal short gill rakers vs. Stn130, the number of distal short gill rakers vs. Stn178, and the anterior, central, posterior and total number of plates vs. Stn381. The same result was obtained after control for neutral F_{ST}. Figure 6 highlights some of the correlations. The relationship

between P_{ST} for the length of the first dorsal spine and F_{ST} at locus Stn9 (*Spine1-a*) was marginally nonsignificant (Fig. 6a; $P = 0.0607$). Some population pairs with relatively high F_{ST} but low P_{ST} blurred the relationship. No outlier F_{ST}s as revealed with the *F*-test in DETSEL were detected at locus Stn9. Equally, no outlier F_{ST}s were detected at locus Stn130 (*Raker#-a*), despite the significant correlation with the divergence in number of frontal short gill rakers (Fig. 6b; $P = 0.0159$). Here, the range of F_{ST} was slightly lower compared to the range of P_{ST}.

Divergence for one trait, the number of distal short gill rakers, was unexpectedly correlated with neutral F_{ST} (Fig. 6c; $P = 0.0107$). However, the correlation between P_{ST} at this trait and F_{ST} at locus Stn178 (*Raker#-b*) was even stronger, showing a bimodal distribution and several outliers (Fig. 6d; $P = 0.0386$ after control for neutral F_{ST}). This was due to fixation of the locus in population S12a and S13a. Both upland populations were characterized by a low number of distal short gill rakers, creating relatively high P_{ST} values with other populations. H_E at locus Stn178 was significantly lower than H_E at neutral loci in both lowland and upland populations (Fig. 5). F_{ST} at Stn178 had a slightly lower range compared to P_{ST} (Fig. 6d). Interestingly, the F_{ST} combining locus Stn178 (*Raker#-b*) and Stn130 (*Raker#-a*) correlated better with P_{ST} at distal short gill rakers ($R = 0.60$; $P = 0.0157$) than the F_{ST} at both loci separately. This was not the case for frontal short gill rakers ($R = 0.03$; $P = 0.4384$). P_{ST} at total (frontal + distal) number of short gill rakers correlated strongly with F_{ST} at *Raker#-a* ($R = 0.54$; $P = 0.0087$), but not with F_{ST} at *Raker#-b*; $R = 0.07$; $P = 0.3320$).

The strongest result was obtained at locus Stn381 (*Eda*). All lowland–upland F_{ST}s were detected as outliers, and there was a good match with P_{ST} at plate number (Fig. 6e; $P = 0.0180$). The distribution of P_{ST} and F_{ST} was also similar (i.e. ranging between 0 and 0.55) and characterized by the same bimodality.

Discussion

Thanks to the increasing understanding of the molecular functioning of phenotypic variation in the three-spined stickleback (Peichel *et al*. 2001; Colosimo *et al*. 2004, 2005; Cresko *et al*. 2004; Shapiro *et al*. 2004), direct comparisons of how natural selection affects quantitative traits and their underlying genes or candidate genes have become possible. So far, relying on well-identified genes for such comparison was only within the scope of classical model systems like *Arabidopsis* (e.g. Le Corre 2005). The strength of the stickleback model lies in the repetitive cases of parallel evolution, which provide strong evidence for the contribution of natural selection to phenotypic evolution (Bell & Foster 1994; Schluter *et al*. 2004). Phenotypic traits and the underlying candidate genes (related to growth) have been screened in populations of just one other model fish for parallel evolution, the lake whitefish (*Coregonus clupeaformis*) species complex (Bernatchez 2004; Rogers & Bernatchez 2005).

Here, we studied the adaptive divergence in a West European lowland–upland stickleback system, a particular case of the anadromous-freshwater system (McKinnon & Rundle 2002). We inferred the role of divergent selection, comparing phenotypic variation at different morphological traits and genetic variation at associated QTL with genetic variation at putative neutral markers. First, we discuss the characteristics of lowland and upland populations, including the phenotypic signals that, assuming minor nonadditive genetic effects and minor environmental effects, point to divergent selection (Merilä & Crnokrak 2001; Storz 2002). Then, we take into account that phenotypic variance may not exclusively represent additive genetic variance, and that P_{ST} values may be inflated. We discuss the potential of confirming phenotypic signals of divergent selection by genetic divergence at the underlying QTL. Confirmation implies that P_{ST} can be interpreted as Q_{ST} (Spitze 1993).

Phenotypic signals of divergent selection

The divergence between lowland and upland sticklebacks is largely similar to the more general anadromous-freshwater divergence (McKinnon & Rundle 2002; McKinnon *et al*. 2004; Schluter *et al*. 2004; Jones *et al*. 2006). The most obvious morphological traits distinguishing lowland and upland sticklebacks were armour traits (lateral plates and dorsal and pelvic spines). Assuming minor nonadditive genetic effects and minor environmental effects, these traits showed signals of divergent selection because of the magnitude of phenotypic divergence (P_{ST}) compared to the expectations based on neutral loci. A trait relevant with respect to the divergence between some lowland and upland populations was short gill raker morphology. Gill rakers are important in the trophic ecology of sticklebacks. Differences in gill raker number have also been reported between lake–stream (Hendry & Taylor 2004) and benthic–limnetic (Schluter 1993) species pairs.

Anadromous sticklebacks have been observed to be larger and more slender than freshwater sticklebacks (McKinnon *et al*. 2004; Schluter *et al*. 2004), or larger and more robustly shaped (Jones *et al*. 2006). We did not observe differences in size or shape among these lowland and upland sticklebacks. Large bodies may have relevance neither in small lowland polder streams, nor in upland freshwater streams. Lowland polder populations may be atypical with respect to body size; populations from nearby estuarine habitats are probably larger (F. Ollevier, personal communication). Assortative mating based on

body size has been observed to account for mating incompatibilities between anadromous and freshwater populations (McKinnon *et al*. 2004).

Do the observed differences of traits among lowland and upland populations have an adaptive basis, suggesting that their divergence may have been driven by natural selection? Alternatively, inflated P_{ST} values could reflect (non)adaptive phenotypic plasticity where trait expression is controlled by environmental effects (Via & Lande 1985). For instance, Day *et al*. (1994) showed that plasticity in stickleback accounted for up to 58% when comparing five foraging-related traits in benthic and limnetic sticklebacks that were fed the natural diet of the other morph. On the other hand, crossing experiments in sticklebacks suggested high additive genetic variance components with h^2-estimates in the laboratory as high as 0.42 (lower gill rakers), 0.33 (upper gill rakers) and 0.37 (lateral plate number) (Hermida *et al*. 2002). Schluter *et al*. (2004) observed parallel inheritance of genetic differences in adaptive traits (lateral plate number and body shape) in two independent lineages of three-spined sticklebacks, suggesting that an ancestral trait in close relatives would follow the same developmental pathway when exposed to similar selection pressures. These results suggest an additive genetic basis for some traits analysed in this study, implying that they could be structured by selection. However, the relevance for sticklebacks around the North Sea remains unknown as these indications originate from South Europe, Asia and North America. In addition, besides additive genetic effects, dominance and epistatic effects may also affect Q_{ST}/P_{ST} estimates. For instance, in the anadromous-freshwater system, plate number is determined by partial dominance (Schluter *et al*. 2004), whose effect on Q_{ST}/P_{ST} estimates is not well understood (Merilä & Crnokrak 2001).

Genetic evidence for divergent selection

The availability of fine-scaled genetic maps, allowing for the assessment of genetic divergence at quantitative trait loci (QTL), represents one way to confirm the indirect phenotypic signals of divergent selection. Only one out of seven QTL pointed to diversifying selection between lowland and upland stickleback populations. Differentiation at locus Stn381, which is closely linked to the *Eda* gene, a major QTL for plate number, exceeded neutral expectations, concordant with the phenotypic signal of divergent selection for this trait. With few exceptions, the correspondence between genotypes at locus Stn381 and plate morphs was identical to Colosimo *et al*. (2005) who used this locus to screen anadromous populations for low-plated alleles. *Eda* provides a uniform background for the independent loss of lateral plates in freshwater populations across almost the full geographical range of the three-spined stickleback. Based on sequence diversity among and within marine and freshwater haplotypes, the causative mutation must be ancient (Colosimo *et al*. 2005). Thus, observing relationships in natural populations between plate morphs and loci linked to *Eda* would seem unlikely (Calafell *et al*. 2001). However, Stn381 is positioned within a ~450-bp intron of *Eda* (intron 6; Colosimo *et al*. 2005), and thus is very tightly linked.

Why do QTL-based F_{ST} values not show evidence of divergent selection for other divergent traits between lowland and upland sticklebacks? F_{ST} values close to neutral levels at a locus cannot be interpreted as evidence against divergent selection at that locus. There are several scenarios under which divergent selection would not be detected. First, a QTL found in one set of populations may not control the trait in another set of populations. In particular, the genetic background of Peichel *et al*.'s (2001) regional benthic–limnetic mapping family is not assumed to be fully representative for our distantly related set of lowland and upland populations. Second, even if the genetic background is representative for our stickleback system, a marker linked to the causative mutation may not show up as an outlier. One reason is the decay of the hitchhiking effect as a result of recombination between the causative nucleotides and the linked marker (Calafell *et al*. 2001). High mutation rates for microsatellite markers may also lead to the loss of the selective signal (Calafell *et al*. 2001). In the case of polygenic traits, the contribution of covariances in allele frequencies among QTL to the divergence of these traits increases as a positive function of QTL number. It complicates the detection of deviations from neutrality at the individual QTL (Latta 2003). Peichel *et al*. (2001) reported one QTL for the first dorsal spine (*Spine1-a*), two QTL for the second dorsal spine (*Spine2-a* and *Spine2-b*) and two for the short gill rakers (*Raker#-a* and *Raker#-b*). If these traits are indeed polygenic, it may explain those cases where, unlike P_{ST}, F_{ST} at corresponding QTL did not exceed neutral levels. In summary, the differentiation at microsatellite markers that are tightly linked to a QTL [e.g. < 5 cM in Peichel *et al*.'s (2001) cross] may not be expected to point to divergent selection between our lowland and upland populations, especially when traits are polygenic. Likewise, phenotypic signals of stabilizing (uniform) selection withinlowland and withinupland populations may be difficult to confirm genetically in most cases, whereas it is straightforward in the case of *Eda* and plate number.

Correlations between QTL-based F_{ST} and P_{ST} over the entire set of population pairs, the presence of outlier F_{ST}s at the level of individual population pairs, and the comparison of ranges of QTL-based F_{ST} and P_{ST} may be indicative of the number of loci underlying the trait or the physical linkage of the QTL. For a tightly linked major locus controlling the trait, as in the case of *Eda*, a strong correlation and

comparable ranges of F_{ST} and P_{ST} are probably not surprising. Instead, for a polygenic trait, the decoupling of Q_{ST}/P_{ST} and QTL-based F_{ST} resulting from covariances in allele frequencies among QTL may affect each population pair in a different way. In particular, with high gene flow and diversifying selection pressure, positive covariances develop within a population pair leading to relatively low QTL-based F_{ST} compared to Q_{ST} (Latta 2003). With restricted gene flow and uniform selection pressure, negative covariances develop leading to relatively high QTL-based F_{ST} compared to Q_{ST}. This may strongly affect the correlation over the entire set of population pairs. Weak physical linkage of the QTL may be another cause of the breakdown of the correlation between both measures. A more technical issue that may influence the decoupling of Q_{ST}/P_{ST} and F_{ST} is the underestimation of F_{ST} when derived from highly polymorphic loci (Hedrick 2005). It is difficult to attribute the lack of correlation to one particular reason. However, among the traits mapped by Peichel *et al*. (2001), we obtained better correlations and more outlier F_{ST}s for QTL having higher LOD scores and percentages of variation explained (PVE) in the original mapping study. For dorsal spine QTL (*Spine1-a*: LOD score 4.7, PVE 21%; *Spine2-a*: LOD score 4.5, PVE 22%; *Spine2-b*: LOD score 3.4, PVE 17%), we observed marginal to no correlations with dorsal spine length P_{ST}, and no outlying QTL-based F_{ST}s. Instead, correlations between pairwise F_{ST} at short gill raker QTL (*Raker#-a*: LOD score 5.5, PVE: 26%; *Raker#*-b: LOD score 6.8, PVE: 37%) and short gill raker P_{ST}s were significant, and outlier F_{ST}s were detected at *Raker#-b*. This suggests that the potential for detecting effects at QTL in natural populations relates to the quality parameters of the original mapping results.

Correlations between QTL-based F_{ST} and P_{ST} were corrected for neutral F_{ST}. This might not be necessary as relationships between variation in quantitative traits and neutral markers are unexpected and generally poor (Reed & Frankham 2001). Nevertheless, they may be observed occasionally as in the case of distal short gill rakers, and may be explained by the following mechanisms (see Merilä & Crnokrak 2001; Leinonen *et al*. 2006). First, selection pressures may fluctuate along a geographical gradient that correlates with neutral divergence when shaped by isolation by distance. Second, under restricted gene flow, drift may affect QTL as long as they are not under strong selection. However, our populations were not characterized by low levels of gene flow in comparison to upstream reference collections. Moreover, strong selection is a straightforward explanation for the significantly lower genetic variability at Stn178, linked to *Raker#-b*, in both lowland and upland populations. Hence, it is possible that variation at distal short gill rakers is influenced by heterogeneity in selection pressures, increasing gradually with geographical distance. The detection of outlier F_{ST}s at the level of individual population pairs suggested the fixation of one short gill raker gene (*Raker#-b*) in S12a and S13a, congruent with their divergent distal short gill raker morphology. It is unclear what differentiates the diet of these populations. The fact that we observed the best correlation between P_{ST} for distal short gill rakers and F_{ST} at combined (*Raker#-a*, *Raker#-b*) rather than single QTL may indicate that this trait is controlled by at least two genes. Numbers of frontal short gill rakers appeared to be affected by both QTL in a different way. We did not observe equivalent patterns for long gill rakers; Peichel *et al*. (2001) suggested that this trait is affected by a large number of other genes.

Conclusion

We combined an indirect approach, identifying traits that are likely targets of selection based on phenotypic divergence (P_{ST}), with a direct test for divergent selection at the underlying QTL. For only one phenotypic trait (lateral plate number) could we rule out the contribution of environmental effects and nonadditive genetic effects to high P_{ST} in the lowland–upland system. Genetic evidence for the adaptive value of short gill rakers was limited to some population pairs, but it was sufficiently strong to rule out a major contribution of neutral processes or phenotypic plasticity. Signals of phenotypic divergence for dorsal and pelvic spines could not be confirmed genetically, and hence the biological mechanism behind the observed phenotypic differences remains inconclusive. This is the reason why we propose to strictly make a distinction between Q_{ST} and P_{ST}. Q_{ST} should rely on breeding designs, whereas the interpretation of P_{ST} as Q_{ST} is only justifiable when levels of phenotypic divergence correspond to levels of divergence at the underlying QTL. The increasing availability of genomic tools necessitates a stricter interpretation.

In general, divergence at quantitative traits and QTL-based F_{ST} in natural populations may be strongly related in the case of a single major gene, decoupled when traits are under polygenic control, or uncorrelated in the case linkage is not preserved. Selection for similar adaptive traits across environments may not necessarily implicate the same genetic basis. The variety and complexity of QTL contributions to phenotype and the impact of genetic background can be considerable (Mitchell-Olds & Schmitt 2006; Sinha *et al*. 2006). Our test of the action of divergent selection showed that linkage between some markers and phenotypic traits can be detected on a continent different from the original mapping families. Before hunting down new candidate genes, screening natural populations for phenotypes and corresponding QTL may reveal which known candidate genes constitute the wider molecular basis of parallel morphological evolution in sticklebacks (Peichel 2005).

Acknowledgements

This paper greatly benefited from comments by L. De Meester, A.P. Hendry, G.E. Maes, C.L. Peichel, A. Vasemägi, and two anonymous referees. We thank B. Christiaen and I. Hontis for field support. J.A.M.R. received a PhD fellowship from the Fund for Scientific Research — Flanders (F.W.O.-Vlaanderen) and M.H.D.L. from the Institute for the Promotion of Innovation by Science and Technology in Flanders (I.W.T.). Research was sponsored by F.W.O.-Vlaanderen (project G.0142.03) and EU NoE Marine Genomics Europe (project no. GOCE-CT-2004-505403).

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This paper represents one of the major common interests of the authors, i.e. the ecological genetics and genomics of fishes. Joost Raeymaekers and Sarah Geldof focus on the ecological genetics of sticklebacks. Jeroen Van Houdt is interested in conservation genetics and genomics. Maarten Larmuseau prepares a PhD on local adaptation in marine fishes combining genomic, phenotypic and environmental information. Filip Volckaert studies the intraspecific evolution of fishes and supervises the team, which is part of the Laboratory of Aquatic Ecology (http://www.kuleuven.be/bio/eco).
