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SPECIAL ISSUE: THE MOLECULAR MECHANISMS OF ADAPTATION AND SPECIATION: INTEGRATING GENOMIC AND MOLECULAR APPROACHES

Genomic landscape of early ecological speciation initiated by selection on nuptial colour

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Abstract

Ecological speciation is the evolution of reproductive isolation as a consequence of direct divergent natural selection or ecologically mediated divergent sexual selection. While the genomic signature of the former has been extensively studied in recent years, only few examples exist for genomic differentiation where environment-dependent sexual selection has played an important role. Here, we describe a very young (~90 years old) population of threespine sticklebacks exhibiting phenotypic and genomic differentiation between two habitats within the same pond. We show that differentiation among habitats is limited to male throat colour and nest type, traits known to be subject to sexual selection. Divergence in these traits mirrors divergence in much older benthic and limnetic stickleback species pairs from North American west coast lakes, which also occur in sympatry but are strongly reproductively isolated from each other. We demonstrate that in our population, differences in throat colour and breeding have been stable over a decade, but in contrast to North American benthic and limnetic stickleback species, these mating trait differences are not accompanied by divergence in morphology related to feeding, predator defence or swimming performance. Using genomewide SNP data, we find multiple genomic islands with moderate differentiation spread across several chromosomes, whereas the rest of the genome is undifferentiated. The islands contain potential candidate genes involved in visual perception of colour. Our results suggest that phenotypic and multichromosome genomic divergence of these morphs was driven by environment-dependent sexual selection, demonstrating incipient speciation after only a few decades of divergence in sympatry.

Keywords: ecological speciation, genomic islands, sexual selection, sympatric divergence, threespine stickleback

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Introduction

Ecological speciation, the evolution of reproductive isolation between groups of individuals due to adaptation to different environments (Rundle & Nosil 2005), has received much attention in the last decade. However, the contributions of different evolutionary forces to the initiation and completion of speciation, their interactions and the chronology in which they operate are not yet well understood. The rise of the genomics era has come with much promise in particular for ecological speciation research (Rice *et al.* 2011; Nosil 2012; Seehausen *et al.* 2014), as targets of divergent selection can be detected at the genome level and insight into the genomic architecture of traits and genomic differentiation may unravel some of the mysteries about why some populations split and others do not, and why

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some lineages speciate more often or more rapidly than others. Consequently, many putative cases of ecological speciation have recently been the subject of genomic study, but most of these either are allopatric or parapatric ecotypes that do not persist in real sympatry (Soria-Carrasco *et al.* 2014) or of species pairs that do persist in sympatry but are already thousands to millions of generations divergent (Jones *et al.* 2012a; Nadeau *et al.* 2012; Renaut *et al.* 2013; Arnegard *et al.* 2014; Malinsky *et al.* 2015). Many of the best documented late stages of ecological speciation with now sympatric species have likely undergone an extended allopatric phase (Jones *et al.* 2012a; Martin *et al.* 2013; Renaut *et al.* 2013), making it sometimes difficult to distinguish between effects of divergent selection and other processes affecting genomic differentiation because these species have complex histories with periods of strong isolation (Cruickshank & Hahn 2014). Until now, only very few studies have characterized genomic differentiation in very young sympatric forms that exchange genes (Michel *et al.* 2010; Malinsky *et al.* 2015).

The early stage of ecological speciation, that is when divergent or disruptive natural or environment-dependent sexual selection initiates reproductive isolation, is of particular interest because barriers reducing gene flow early in the speciation process have a larger effect on the origin of reproductive isolation than late-acting barriers (Coyne & Orr 2004). Very early stages may, for instance, be needed to investigate the relative importance of divergent natural and sexual selection in initiating divergence. This is because in most advanced stages of speciation both types of selection have already been acting and may have led to character divergence, making it impossible to tell how the process began (Maan & Seehausen 2011). The beginning of ecological speciation or 'incipient' speciation is thought to be accompanied by genomic divergence in multiple small genomic regions diverging despite gene flow (Wu 2001; Feder *et al.* 2012; Marques *et al.* 2016), a genomic signature of divergent selection reducing gene flow locally in the genome and therein causing 'isolation by adaptation' (Nosil *et al.* 2008; Nosil 2012).

Here, we characterize a case of very recent phenotypic and genomic divergence in sympatry observed within a population of threespine stickleback (*Gasterosteus aculeatus* species complex) in a clearwater pond, the Jordeweiher, near Bern, Switzerland. Stickleback have colonized the artificial Jordeweiher pond not more than 90 years ago. The population is now polymorphic for many traits that differ among sympatric limnetic and benthic stickleback species from lakes on the west coast of Canada (McPhail 1994; Vines & Schluter 2006), including nest type, breeding habitat, male throat colour, body shape and size. This variation in phenotypic traits may have been facilitated by a hybrid origin of the population: the Jordeweiher was colonized by stickleback from an extensive hybrid zone between divergent stickleback lineages from western, northern and eastern Europe that is situated in central Switzerland and formed within the last 150 years (Lucek *et al.* 2010; Roy *et al.* 2015). Jordeweiher stickleback (population 'EYM' in Roy *et al.* 2015) show the typical mitochondrial haplotype composition of Central Swiss populations, consisting of Rhine (Northern) and Baltic (Eastern) haplotypes (population 'EYM' in Roy *et al.* 2015; K. Lucek & O. Seehausen, unpublished data). Additionally, haplotypes from the Rhone lineage were found in Lake Wohlen just 1.5 km downstream from the Jordeweiher (Lucek *et al.* 2010).

Stickleback in this ~3200 m^2 spring-fed clearwater pond build nests in two distinct but directly adjacent habitats that differ in multiple biotic and abiotic factors: 'offshore' habitat, the open, flat floor covered in fairly stable but soft sediment of very light colour (Fig. 1a), and 'nearshore' habitat, the steep clay bank below overhanging trees with increased structural complexity (branches, tree roots, leaves, Fig. 1d). Besides substrate, slope and habitat complexity, the habitats also differ in light regime: offshore habitat receives direct and strong vertical sunlight throughout most of the day and the sediment reflects brightly, while nearshore habitat is characterized by a more heterogeneous and dynamic light mosaic due to shade from overhanging trees, and the floor is covered in much darker leaf litter (Fig. 1a, d). Furthermore, the habitats may also differ in predator composition: only two avian predators have been recorded on the pond, none of which is likely to reach down to the bottom in the deeper offshore habitat, common kingfishers (*Alcedo atthis*) and grey herons (*Ardea cinerea*). Neither of them breeds in the nearest vicinity, and they are thus only occasional visitors. The impoverished predator fauna is indeed a unique feature of the Jordeweiher compared to other stickleback habitats in Switzerland: only invertebrate predators such as large dragonfly larvae (suborder Anisoptera) are moderately abundant (Zeller *et al.* 2012), while a single northern pike (*Esox lucius*) was the only fish predator repeatedly observed in a single year. This low predation pressure could have allowed stickleback to colonize most of the available pond habitats, including the open pond with little shelter.

In 2007, OS discovered that variation in male nuptial colour, body shape and nest morphology, an extended phenotype (Hunter 2009) shown by breeding males, may be associated with these habitats. This would be an example of multidimensional differentiation between phenotypes that may have evolved in sympatry, not known from stickleback anywhere else in central Europe. In this study, we quantify phenotypic, ecological and genomic

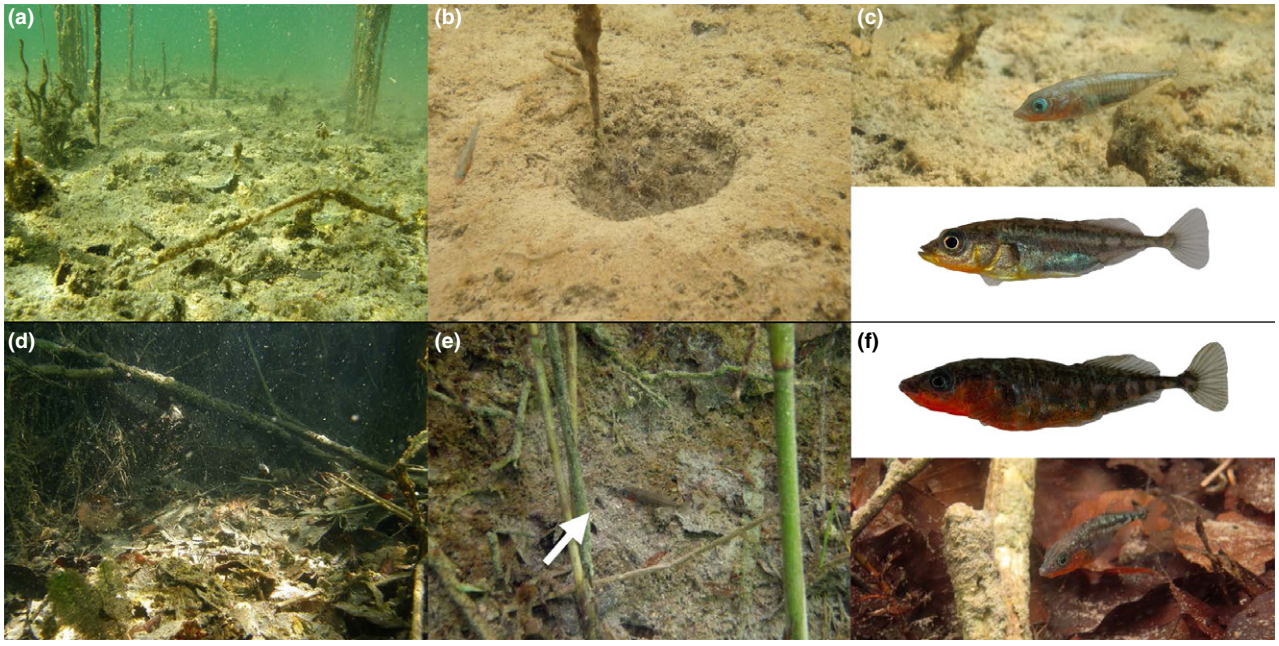

Fig. 1 Threespine stickleback breed in two divergent habitats, 'offshore' (a–c) and 'nearshore' (d–f), in the Jordeweiher pond near Bern, Switzerland. While offshore habitat (a) consists of an open, flat, muddy floor, with direct sunlight and greater depth down to 3 m, nearshore habitat (d) is a steep clay bank below overhanging trees producing a more heterogeneous and dynamic light mosaic and a more complex habitat with branches, tree roots and leaves. Stickleback males breeding in offshore habitat (c) have an orange throat and pale body colour and build large, deep crater nests (b), while nearshore breeding males (f) have a red throat and a darker body with more dark pigments and build concealed nests (e).

differentiation between males of the different colour morphs and between males from different breeding habitats and we ask whether feeding-related, defence-related or sexual/social signalling traits are more strongly differentiated. We then investigate genomic differentiation and identify genomic islands diverging between male colour morphs and between males from different habitats. Finally, we identify genomic candidate targets for divergent selection between colour morphs and habitats. Based on the kind of traits showing phenotypic divergence between distinct breeding habitats, we infer the likely involvement of environment-dependent sexual selection. Therefore, we aimed to uncover the genomic landscape of very early ecological speciation driven by environment-dependent sexual selection, which has not yet been studied in contrast to the genomics of ecological speciation largely driven by natural selection such as selection on resource use or predator avoidance.

Methods

Sampling site and collection

The Jordeweiher pond near Wohlen, Bern, Switzerland (46°57′24″ N, 7°23′21″ E), was built between 1901 and 1931 (Stengel & Lutz 1901; swisstopo 2015). We collected male stickleback from the pond in four different years: 2007 (June 12, $n = 20$), 2012 (May 6, $n = 79$), 2013 (July 18–23, $n = 21$) and 2015 (May 18–25, $n = 20$). In 2007 and 2012, we used minnow traps to collect fish, whereas in 2013 and 2015, we captured breeding males at their nests with hand nets while scuba-diving. Upon capture, males were immediately photographed in a cuvette and subsequently anesthetized and euthanized using a clove oil solution, except for males in 2015, which were also tested in mate choice and nest site choice experiments (Feller *et al.* 2016). Fish capture and euthanasia were in accordance with the Swiss fisheries and veterinary legislation and granted permits issued by the cantonal veterinary office in Bern (permit numbers BE66/13, BE7/15) and by the owner of the Jordeweiher fishery rights (Augsburger AG, Hinterkappelen, Switzerland). In addition, between April and August 2008, we surveyed the population by snorkelling and photographing. We marked and mapped nest locations in the field in 2008 and triangulated and digitally mapped nest locations in 2013 and 2015 with QGIS v2.6.1 (QGIS Development Team 2015). We measured water depth at nest locations in 2013 and 2015 as well as the following nest characters for complete nests in 2013: diameter, slope, presence of assembled vegetation, presence and depth of depression and openness vs. concealment. Based on the slope and substrate where the stickleback built their nests, we classified the pond

habitat into two breeding habitat categories: the 'offshore' habitat characterized by a thick layer of accumulated mud substrate and a flat topography (inclination <15°), and the 'nearshore' habitat, characterized by clay-like substrate without accumulating loose substrate but covered in leave litter and a steep topography (inclination > 15°, Fig. 3).

Colour analysis

We measured male throat coloration from cuvette photographs taken in front of a neutral grey card. Males were photographed in ambient light in 2007 and 2012 and in standardized light from two external flashes in a black velour-coated box in 2013 and 2015, using a Nikon E8700 in 2007 and a Canon EOS 7D in 2012–2015. Photographs were colour-standardized in PHOTOSHOP LIGHTROOM v3.6 (Adobe Inc.) using the neutral grey background for automatic white balance adjustment, and male throat coloration was measured in a 1 mm^2 circle without melanophores below the eye (Fig. S1, Supporting information) using IMAGEJ v1.49 (Schneider *et al.* 2012). The median red, blue and green (RGB) values from these sampled pixels were transformed into a median hue angle for each male (Preucil 1953; see also Feller *et al.* 2016), hereafter 'throat colour'. Because not all males had attained their full nuptial colours in some years and because time in minnow traps may have caused males to lose colour intensity in 2012 (Fig. 2c), one observer (DM) assigned the photographs to three nuptial coloration expression levels: 'fully coloured' males showed excessive yellow to red coloration on throat and sides of the head up to the operculum, 'pale' males displayed the same distribution of colours as fully coloured males, but with a lower intensity, while 'throat-only' males showed coloration restricted to the lower throat, reflecting pre- or postbreeding condition.

We tested the distribution of throat colour in the population for multimodality and assigned males to the respective modes using a cluster analysis based on a Gaussian mixture model implemented in the R-library *mclust* (Fraley & Raftery 2002). The *mclust* algorithm fits mixture models with varying numbers of normal mixture components to the data using the EM algorithm (Fraley & Raftery 2002). We assumed both equal and unequal variances for each mixture component, with equal variance models showing a better model fit judged by the Bayesian information criterion (BIC). We fitted up to three mixture components to the data and performed likelihood ratio tests (LRTs) to find the best model, with significance estimated from 10 000 bootstrap LRT statistics. Based on the best fitting model, *mclust* assigned males to two clusters referred to as, a 'red' and an 'orange' cluster, corresponding to the two mixture components and hence the two modes in the throat colour distribution.

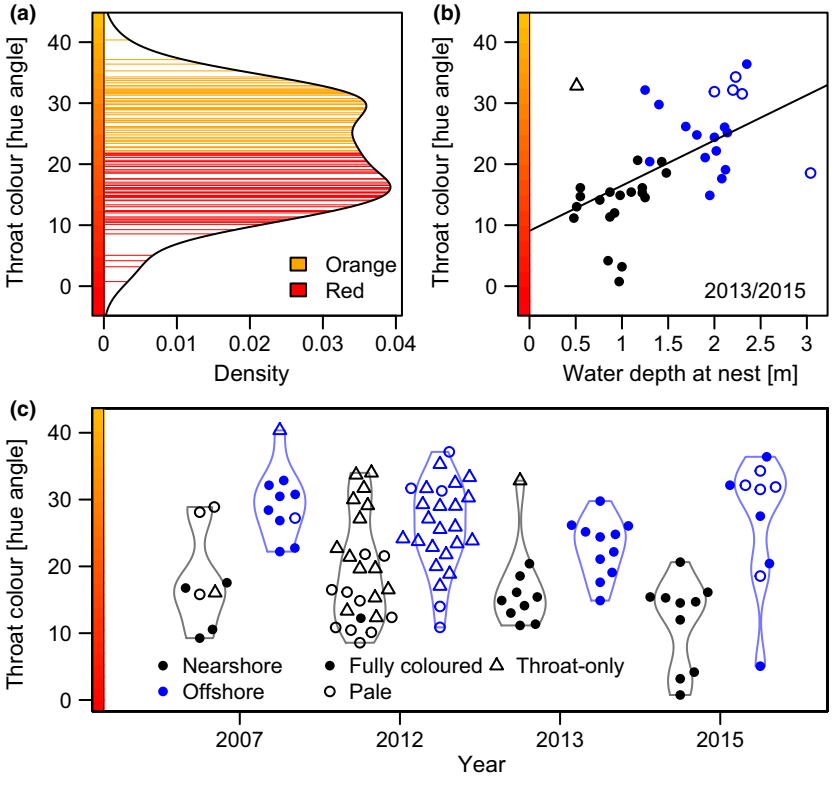

Fig. 2 Bimodal distribution of throat colour and phenotype–environment association in Jordeweiher threespine stickleback. (a) Throat colour distribution and cluster analysis assignment of each male (coloured vertical bars) to the two supported 'red' and 'orange' clusters. (b) The phenotype–environment association is significant using both continuous variables (hue, depth, males from 2013 to 2015 only, see text for statistics) and (c) discrete habitat categories (blue dots: offshore, black dots: nearshore), the latter demonstrating temporal stability of the throat colour vs breeding habitat association for at least 9 years. Symbols show the intensity of nuptial coloration: Males sampled in 2012 showed more faded nuptial coloration, likely due to the early sampling date and the capture using minnow traps.

We tested for a phenotype–environment association between breeding males' throat colour and breeding habitat. We first used throat hue angle and water depth at the nest (2013 and 2015 males only) in a linear mixed-effect model with colour as response variable, depth as predictor variable and sampling year as random effect. To test for temporal stability of the throat colour and habitat association, we included males from 2007 and 2012 and substituted depth by the binary 'nearshore'/'offshore' habitat category in the linear mixed-effect model.

Linear and geometric morphometrics

We measured 17 standard linear morphological traits and placed 19 landmarks (Fig. S1, Supporting information) to study morphological variation among Jordeweiher stickleback males in linear and shape traits, using TPSDIG v2.17 (Rohlf 2015), MORPHOJ 1.06d (Klingenberg 2011) and custom R scripts. We size-corrected both linear and geometric morphometric data by extracting residuals from linear regressions of single traits and Procrustes coordinates, respectively, against standard length. We tested whether male breeding habitat and colour morph can be predicted by morphometric distances or shape traits using linear mixed-effect models, with traits as predictors and sampling year as random effect. We tested standard length, all size-corrected linear traits separately and combined into principal components (the five leading axes) as well as the first five principal components of overall shape, head and body shape using false-discovery rate-adjusted *P*-values to assess significance of predictors. Following the approach by Kaeuffer *et al.* (2012), we calculated P_{ST}, a scale-free estimator of phenotypic differentiation analogous to F_{ST}, for standard length, each size-corrected trait, for each of the first three principal components combining either all size-corrected traits, feeding morphology, antipredator defence morphology or swimming performance traits (see Fig. S1, Supporting information for grouping), and for each of the three-first principal components of shape traits (whole body, head and body shape, respectively), between males grouped by colour morph and by breeding habitat. By bootstrapping the data 1000 times, we tested for significant differentiation among the groups, that is whether the 95% confidence interval for a P_{ST} exceeded zero, using bootstrap *P*-values adjusted for multiple testing using the false-discovery rate method (Benjamini & Hochberg 1995).

Stomach content and stable isotope analyses

Stomach contents of stickleback collected in 2007 were analysed under a dissecting microscope, and we identified organisms in the diet to the level of order or family following Lucek *et al.* (2012). We calculated the proportion of planktonic prey, that is the ratio of *Copepoda* plus *Cladocera* over the total number of food items. For stable isotope analysis, muscle tissue from the 2007 males was dried in an oven at 75 °C for 48 h, pulverized, weighed to 0.25–0.28 mg packed into tin capsules and sent to the Environmental Isotope Laboratory (University of Waterloo, ON, Canada), as described in Lucek *et al.* (2013). We tested whether male breeding habitat and colour morph can be predicted by $\delta^{13}C$ and $\delta^{15}N$ isotope ratios or the percentage of planktonic prey using linear mixed-effects models, with isotope ratios and planktonic prey proportion as predictors and sampling year as random effects. Analogous to P_{ST} outlined above, we calculated 'E_{ST}', a measure of ecological differentiation (Kaeuffer *et al.* 2012), for the percentage of planktonic prey and the $\delta^{13}C$ and $\delta^{15}N$ isotope ratios between male colour morphs and breeding habitats and determined significance by bootstrapping the data 1000 times.

Genomic data preparation

We sequenced 21 and 20 Jordeweiher males from 2013 and 2015 using the restriction site-associated DNA (RAD) sequencing protocol by Baird *et al.* (2008), with modifications described in Marques *et al.* (2016). Three RAD libraries were single-end sequenced on an Illumina HiSeq 2000 at the Next Generation Sequencing (NGS) Platform, University of Bern, Switzerland, and the Center of Integrative Genomics (CIG), University of Lausanne, Switzerland. Each library was run on a single lane together with other stickleback samples and 10% bacteriophage PhiX genomic DNA (Illumina Inc., San Diego CA, USA). The three libraries yielded 175, 188 and 142 million 100-bp long reads, respectively. We removed PhiX-reads from raw sequencing reads by alignment to the PhiX reference (Accession no.: NC_001422; Sanger *et al.* 1977), demultiplexed individuals and filtered for an intact *SbfI* restriction site using process_radtags v1.26 (Catchen *et al.* 2011). We aligned stickleback reads against a reassembly of the stickleback genome (Glazer *et al.* 2015) using BOWTIE 2 v2.2.6 (Langmead & Salzberg 2012) with default parameter end-to-end alignment. As described in Marques *et al.* (2016), we recalibrated base quality scores using the PhiX-reads to empirically estimate sequencing error with the GATK v2.7 tools BaseRecalibrator and PrintReads (McKenna *et al.* 2010).

We called variants and genotypes simultaneously using the GATK tool UnifiedGenotyper (McKenna *et al.* 2010), with the following parameters: base quality score minimum 20, SNPs and indel genotype likelihood

model, contamination rate 3%. Using VCFTOOLS v1.1.14 (Danecek *et al.* 2011) and custom python scripts, we removed sites with quality below 30, with more than 50% missing genotypes, indels and sites 3-bp upstream or downstream of indels, SNPs with more than 2 alleles and individuals with more than 40% missing data. We also removed genotypes with quality below 30 and depth below 30 reads. Additionally, we excluded sites on the sex chromosome XIX from the data set, due to uncertainty in mapping and variant calling, as no Y-chromosome reference is available for stickleback yet. Furthermore, we converted heterozygote genotypes with a strong read count imbalance for the two alleles, meaning genotypes with less than 25% reads of the rarer allele, to homozygotes for the more common allele in order to prevent incorrect heterozygote calls due to potential PCR-induced errors. For the detection of genomic islands, we applied a minor allele frequency cut-off of 20% and computed *F*-statistics incorporating an inbreeding term, to prevent effects of potential erroneously called homozygotes due to PCR duplicates present in single-end RAD sequencing data (Baxter *et al.* 2011; Davey *et al.* 2011, 2013; Andrews & Luikart 2014; Puritz *et al.* 2014; Marques *et al.* 2016). We used custom bash and python scripts for filtering steps as well as PGDSPIDER v2.0.9.0 (Lischer & Excoffier 2012) for file conversion.

Population genomic analyses

We computed *F*-statistics (F_{ST}, F_{IT} and F_{IS}) for all Jordeweiher males grouped by colour morph (orange vs. red) or breeding habitat (near- vs. offshore), using a locus-by-locus AMOVA as implemented in ARLEQUIN v3.5.2.3 (Excoffier & Lischer 2010), allowing for within-individual variation and thus inbreeding. We ran 16 000 permutations to assess whether single-locus F_{ST}'s are greater than zero, as suggested by Guo & Thompson (1992). To identify genomic islands of differentiation, defined here as genomic regions with an accumulation of loci with elevated differentiation, we used a hidden Markov model (HMM) approach (Hofer *et al.* 2012; Soria-Carrasco *et al.* 2014; Marques *et al.* 2016). First, we normalized F_{ST} values by transforming to $\log_{10}(F_{ST} + 1)$ and applied an HMM with three normally distributed states to this series of transformed F_{ST} values, corresponding to 'genomic background' differentiation, 'low' and 'high' differentiation, the latter being 'genomic islands of differentiation' and referred to simply as 'genomic islands' from now onwards. Second, we retained genomic islands as such only if they contained loci with statistically significant differentiation after correction for multiple testing, as assessed based on *P*-values from AMOVA permutations corrected for a false-discovery rate of 0.05, following Sun & Cai (2009), Wei *et al.* (2009) and Hofer *et al.* (2012).

To detect putative signatures of selection, we calculated nucleotide diversity in nonoverlapping windows spanning multiple RAD loci, so that a window contained at least 1500 sequenced base pairs (max. 1802 bp) without splitting RAD loci across windows. We used only sites with maximal 50% missing data per group, grouped by colour morph (orange vs. red) or breeding habitat (nearshore vs. offshore). This resulted in 1823 and 1825 windows for males grouped by habitat and colour morph, respectively, spanning along chromosomes a mean distance of 217 kb (median 181 kb, range 37–1773 kb) and 218 kb (median 192 kb, range 29–2159 kb), respectively. We used ARLEQUIN v3.5.2.3 (Excoffier & Lischer 2010) to estimate nucleotide diversity (π) for each group and window and calculated the differences in nucleotide diversity between groups ($\Delta\pi_{nearshore-offshore}$ and $\Delta\pi_{red\text{-}orange}$) for each window. We overlaid the positional information for genomic islands with these windows and assigned them accordingly to 'island windows' if they overlapped with genomic islands or to 'genomic background windows' otherwise. We tested whether the absolute values of $\Delta\pi_{nearshore-offshore}$ and $\Delta\pi_{red\text{-}orange}$ of island windows were different from genomic background windows, using *t*-tests and false-discovery-rate-adjusted *P*-values.

We overlaid positional information for genomic islands with those of Ensembl predicted genes (Jones *et al.* 2012b) and with previously identified quantitative trait loci (QTL), candidate genes, expression outliers and outlier regions (Peichel *et al.* 2001; Colosimo *et al.* 2004, 2005; Cresko *et al.* 2004; Shapiro *et al.* 2004; Kimmel *et al.* 2005; Coyle *et al.* 2007; Miller *et al.* 2007, 2014; Albert *et al.* 2008; Makinen *et al.* 2008a,b; Chan *et al.* 2009, 2010; Kitano *et al.* 2009, 2010, 2013; Hohenlohe *et al.* 2010; DeFaveri *et al.* 2011; Greenwood *et al.* 2011, 2012, 2013, 2015; Shimada *et al.* 2011; Deagle *et al.* 2012; Jones *et al.* 2012a,b; Kaeuffer *et al.* 2012; Malek *et al.* 2012; Rogers *et al.* 2012; Wark *et al.* 2012; Arnegard *et al.* 2014; Berner *et al.* 2014; Cleves *et al.* 2014; Erickson *et al.* 2014, 2015, 2016; Glazer *et al.* 2014, 2015; Liu *et al.* 2014; Terekhanova *et al.* 2014; Yoshida *et al.* 2014; Conte *et al.* 2015; Ellis *et al.* 2015; Feulner *et al.* 2015; Guo *et al.* 2015; Roesti *et al.* 2015; Yong *et al.* 2015; Marques *et al.* 2016). We tested whether the set of genes overlapping with genomic islands was enriched for gene ontology (GO) terms using the STRING v9.1 database (Franceschini *et al.* 2013) with a Bonferroni-corrected alpha level of 0.05. We also tested whether genomic islands fell more often into QTL for 39 trait groups than expected by chance using a permutation approach (Marques *et al.* 2016). Genomic data analysis was performed using the bioinformatics infrastructure of the Genetic Diversity Centre

(GDC), ETH Zurich/Eawag, on the Euler computer cluster at ETH Zurich and on the Ubelix computer cluster at University of Bern, Switzerland. Statistical analyses were conducted in R v3.2.2 (R Development Core Team 2015).

Results

Throat colour polymorphism is stable and associated with the environment

Breeding males in the Jordeweiher pond show a bimodal distribution of throat colour variation, with one mode of red-throated males and another mode of orange-throated males (Fig. 2a, b, LRT statistic = 7.82, $P = 0.022$). Red-throated males predominantly breed in the steep shore part of the pond, the 'nearshore' habitat, while orange-throated males mostly breed on the deeper and flatter bottom of the pond, the 'offshore' habitat (Fig. 3; males 2013 and 2015: $\beta_{\text{water depth at nest}} = 7.41$, $t_{2,36} = 4.00$, $P < 0.001$, males 2007, 2012, 2013 and 2015, $\beta_{\text{habitat}} = 8.98$, $t_{2,104} = 6.70$, $P < 0.001$). This association results in significant phenotypic differentiation between nearshore and offshore males for throat coloration ($P_{ST} = 0.37$, $P < 0.001$, Fig. 4). Furthermore, the association of male throat coloration with breeding habitat persisted over the surveyed period between 2007 and 2015 (Fig. 2c), demonstrating the temporal stability of this phenotype–environment association.

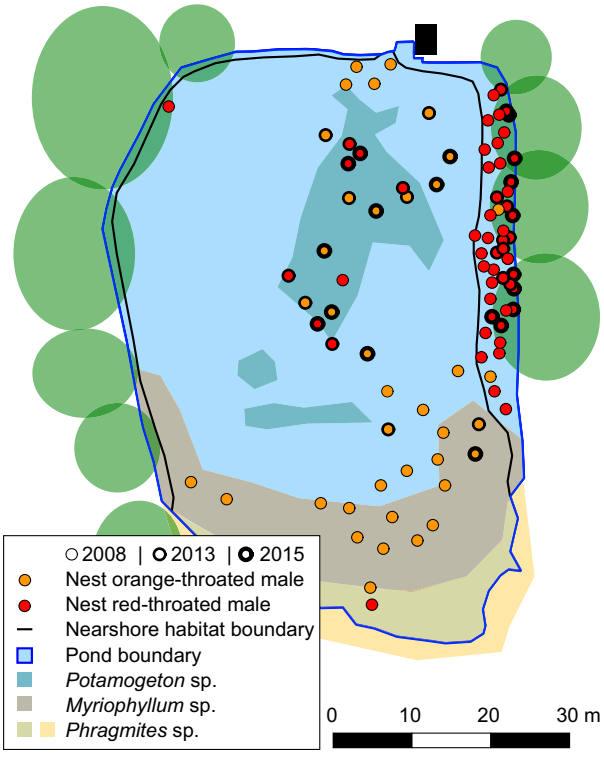

Fig. 3 Distribution of nests in the Jordeweiher across breeding habitats. Steep nearshore habitat, where predominantly red-throated males build their nests, is mostly found at the eastern side of the pond. The flat offshore habitat covers most of the pond bottom, where mostly orange-throated males breed.

Weak differentiation in defence and feeding morphology and ecology

Besides throat coloration, morphological differentiation is weak between red and orange or nearshore- and offshore-breeding males: Red/nearshore males are slightly larger than orange/offshore males, and have slightly larger heads and upper jaws, a shorter second spine and a longer dorsal fin as well as a deeper body (Table 1). However, only swimming performance-related trait differences (body depth and shape, dorsal fin length), predominantly among the colour morphs, remain significant after correction for multiple testing (Table 1, Fig. S2 & S3, Supporting information). Concordantly, morphological differentiation is not significant in any of those traits after correction for multiple testing, neither between habitats nor between colour morphs (Fig. 4).

Estimates of differentiation in feeding ecology among males breeding in different habitats ($\delta^{15}N$ $E_{ST} = 0.11$, $\delta^{13}C$ $E_{ST} = 0.12$, Fig. 4, Table 1) suggest a slight but not significantly increased carbon depletion in offshore-breeding males and an on average slightly elevated trophic position for nearshore males (Figs 4 and S4, Supporting information). This trend is not present among colour morphs. Weak differentiation in morphological traits is similar in direction between both habitats and colour morphs, but slightly stronger among colour morphs (standard length, body depth, swimming performance linear morphology), while ecological differentiation estimates are higher between habitats than between colour morphs (Fig. 4). The degree of differentiation in all these phenotypic and ecological traits is much lower than differentiation in throat coloration (Fig 4).

Genomic islands of differentiation

We studied patterns of genomic differentiation and diversity using a RAD sequencing-derived data set of 2 907 120 sequenced sites passing quality filters, including 11 733 SNPs, distributed across the genome. We computed relative differentiation (F_{ST}) for each SNP between male colour morphs and between males breeding in the two different habitats, using a locus-by-locus AMOVA (see Methods). Averaged across all SNPs, mean genomic differentiation among nearshore- and offshore-breeding males (mean $F_{ST} = -0.0018$,

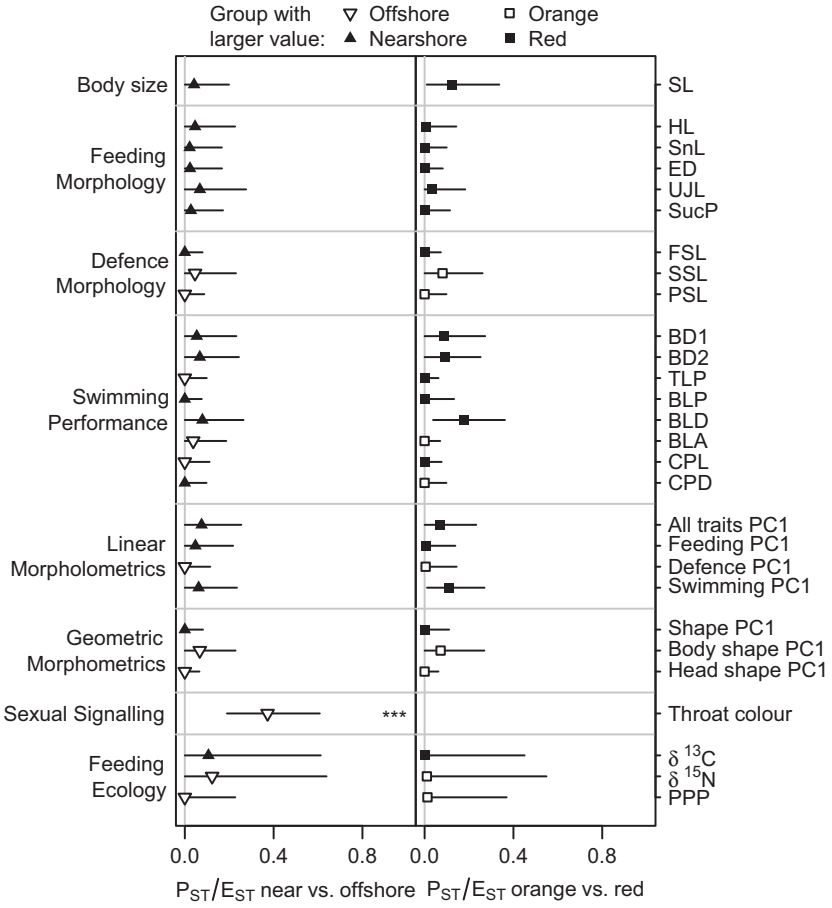

Fig. 4 Phenotypic (P_{ST}) and ecological (E_{ST}) differentiation between stickleback males grouped by breeding habitat and colour morph. Habitat differentiation is only significant for throat colour, a sexual signal, while no differentiation is present in morphological traits associated with feeding, defence and swimming performance. Filled and empty symbols indicate groups with higher absolute or residual values for raw and size-corrected traits, respectively. See Table 1 for trait abbreviations. Whiskers indicate 95% confidence intervals from 1000 bootstrap permutations for P_{ST} and E_{ST} (feeding ecology) estimates. Asterisks indicate significant P_{ST} estimates (***: $P < 0.001$).

permutation test $P > 0.05$) and red- and orange-throated males (mean $F_{ST} = -0.0010$, $P > 0.05$) is not significant and thus there is no genomic background differentiation among them. However, differentiation is heterogeneous across the genome, revealing a number of genomic regions with considerable differentiation ranging up to $F_{ST} = 0.46$ between colour morphs and $F_{ST} = 0.48$ between breeding habitats (Fig. 5b, d). We used a hidden Markov model (HMM) approach and a subset of 7669 SNPs with minor allele frequency >20% to identify regions with an accumulation of differentiated loci. We found 14 such genomic islands of differentiation between red and orange stickleback males and 9 genomic islands between males grouped by breeding habitat (Fig. 5b, d, Table 2). Three genomic islands on chromosomes XII, XIV and XVIII are divergent both between males breeding in different habitats and males of the different colour morphs.

In several genomic islands, nucleotide diversity is reduced in one of the two male types, indicative of habitat- or colour morph-specific selective sweeps in those regions (Fig. 5a, c). For example, island H.21b (Table 1, Fig. 5) shows a highly positive $\Delta\pi_{nearshore-offshore}$, suggesting a reduction in diversity due to a sweep in offshore males. In contrast, island HC.18 shows negative values for both $\Delta\pi_{nearshore-offshore}$ and $\Delta\pi_{red-orange}$ and thus reduced diversity in nearshore/red males, suggesting a selective sweep in nearshore/red males. Among males breeding in different habitats, island H.11 shows decreased diversity in offshore males and island H.16 in nearshore males, while among red and orange males, islands C.2b and HC.12 show low diversity in orange males and island C.20d in red males (Fig. 5). Overall, differences in nucleotide diversity between nearshore vs. offshore males and red vs. orange males, respectively, were higher in genomic islands than in the genomic background (mean island $|\Delta\pi_{nearshore-offshore}| = 3.5 * 10^{-4}$, mean background $|\Delta\pi_{nearshore-offshore}| = 2.2 * 10^{-4}$, $t_{2,45} = -2.66$, $P = 0.011$; mean island $|\Delta\pi_{red-orange}| = 3.4 * 10^{-4}$, mean background $|\Delta\pi_{red-orange}| = 2.1 * 10^{-4}$, $t_{2,60} = -3.17$, $P < 0.001$). At the same time, raw estimates of nucleotide diversity are not lower in genomic islands than in the genomic background, neither within individuals grouped by habitat (mean island $\pi_{nearshore} = 1.37 * 10^{-3}$, mean background $\pi_{nearshore} = 1.41 * 10^{-3}$, $t_{2,47} = -0.31$, $P = 0.753$, mean island $\pi_{offshore} = 1.40 * 10^{-3}$, mean background $\pi_{offshore} = 1.38 * 10^{-3}$, $t_{2,48} = -0.20$,

Table 1 Linear mixed-effects model results for morphological and ecological traits, with summary statistics given for the predictors' habitat and colour, respectively. Significant traits/models after correction for multiple testing are highlighted in bold

		Habitat			Colour		
Trait	Abbr.	β_{trait}	$t_{2,55}$*	*P*-value	β_{trait}	$t_{2,55}$*	*P*-value
Standard length	SL	1.785	2.221	0.030	**2.389**	**2.938**	**0.005**
Head length	HL	0.251	2.134	0.037	0.192	1.554	0.126
Snout length	SnL	0.097	1.521	0.134	0.048	0.742	0.461
Eye diameter	ED	0.078	1.576	0.121	0.039	0.771	0.444
Upper jaw length	UJL	0.148	2.404	0.020	0.124	1.924	0.060
Suction index proxy	SucP	0.307	2.033	0.047	0.295	1.870	0.067
First spine length	FSL	0.034	0.370	0.713	0.004	0.049	0.961
Second spine length	SSL	0.158	1.958	0.055	0.220	2.773	0.008
Pelvic spine length	PSL	0.054	0.510	0.612	0.059	0.545	0.588
Body depth 1	BD1	0.298	2.386	0.021	**0.371**	**2.930**	**0.005**
Body depth 2	BD2	0.337	2.783	0.007	**0.381**	**3.064**	**0.003**
Total length pelvic fin	TLP	0.070	0.689	0.494	0.020	0.185	0.854
Basal length pelvic fin	BLP	0.018	0.403	0.689	0.012	0.261	0.795
Basal length dorsal fin	BLD	**0.282**	**2.973**	**0.004**	**0.363**	**3.838**	**<0.001**
Basal length anal fin	BLA	0.212	1.820	0.074	0.016	0.129	0.898
Caudal peduncle length	CPL	0.077	0.652	0.517	0.018	0.153	0.879
Caudal peduncle depth	CPD	0.027	0.879	0.383	0.011	0.348	0.729
All linear traits PC1	–	**0.665**	**2.952**	**0.005**	**0.723**	**3.118**	**0.003**
Feeding traits PC1	–	0.436	2.377	0.021	0.382	1.981	0.053
Defence traits PC1	–	0.099	0.747	0.458	0.148	1.127	0.265
Swimming traits PC1	–	0.495	2.674	0.010	**0.621**	**3.330**	**0.002**
Throat colour	–	**10.936**	**6.078**	**<0.001**	–	–	–
Head + body shape PC2	–	0.003	0.536	0.594	0.008	1.418	0.162
Body shape PC1	–	0.018	2.708	0.009	**0.022**	**3.365**	**0.001**
Head shape PC1	–	0.000	0.054	0.957	0.007	0.931	0.356
δ^{13}carbon	δ^{13}C	1.948	1.650	0.127	1.397	1.055	0.314
δ^{15}nitrogen	δ^{15}N	0.764	1.570	0.145	0.443	0.803	0.439
Proportion of planktonic prey	PPP	0.074	0.449	0.659	0.185	1.100	0.288

*$t_{2,11}$ for δ^{13}C/δ^{15}N and $t_{2,16}$ for PPP.

$P = 0.84$) nor by colour morph (mean island $\pi_{red} = 1.38 * 10^{-3}$, mean background $\pi_{red} = 1.49 * 10^{-3}$, $t_{2,64} = -1.11$, $P = 0.271$, mean island $\pi_{orange} = 1.39 * 10^{-3}$, mean background $\pi_{orange} = 1.43 * 10^{-3}$, $t_{2,63} = -0.38$, $P = 0.702$). This suggests that genomic islands are likely arising from divergent selection between habitats and colour morphs and not due to older sweeps predating the colonization of the Jordeweiher pond or due to other processes such as background selection (Cruickshank & Hahn 2014; Burri *et al.* 2015), which would instead reduce diversity in both groups at the same genomic regions.

We screened the gene content of genomic islands and found 847 overlapping genes, including 615 genes with orthologues in zebrafish (*Danio rerio*). We did not find enrichment for gene ontology categories among these 847 genes, but we identified a number of putative candidate genes with functions derived from zebrafish phenotypes (Howe *et al.* 2013) that are relevant to the observed phenotypic divergence among Jordeweiher males. The set of overlapping genes contained multiple genes with a role in visual perception, eye, retina and photoreceptor development, photoreceptor maintenance and recovery, genes controlling erythrocyte development responsible for red pigmentation, melanocyte development and iridophore development responsible for blue coloration. Those genes are distributed across multiple genomic islands found in this study, with many islands containing candidate genes involved in both visual system and in pigmentation, which could be possible targets of divergent selection (e.g. island C.2a, C.3, H.11, C.20c, H.21a, Table S1, Supporting information).

The genomic islands overlap with 151 previously identified QTL controlling morphology associated with feeding ecology, body shape and predator defence (Table S2, Supporting information). However, the overlap between QTL and genomic islands is not significantly higher than expected if the islands were randomly distributed across the genome (permutation

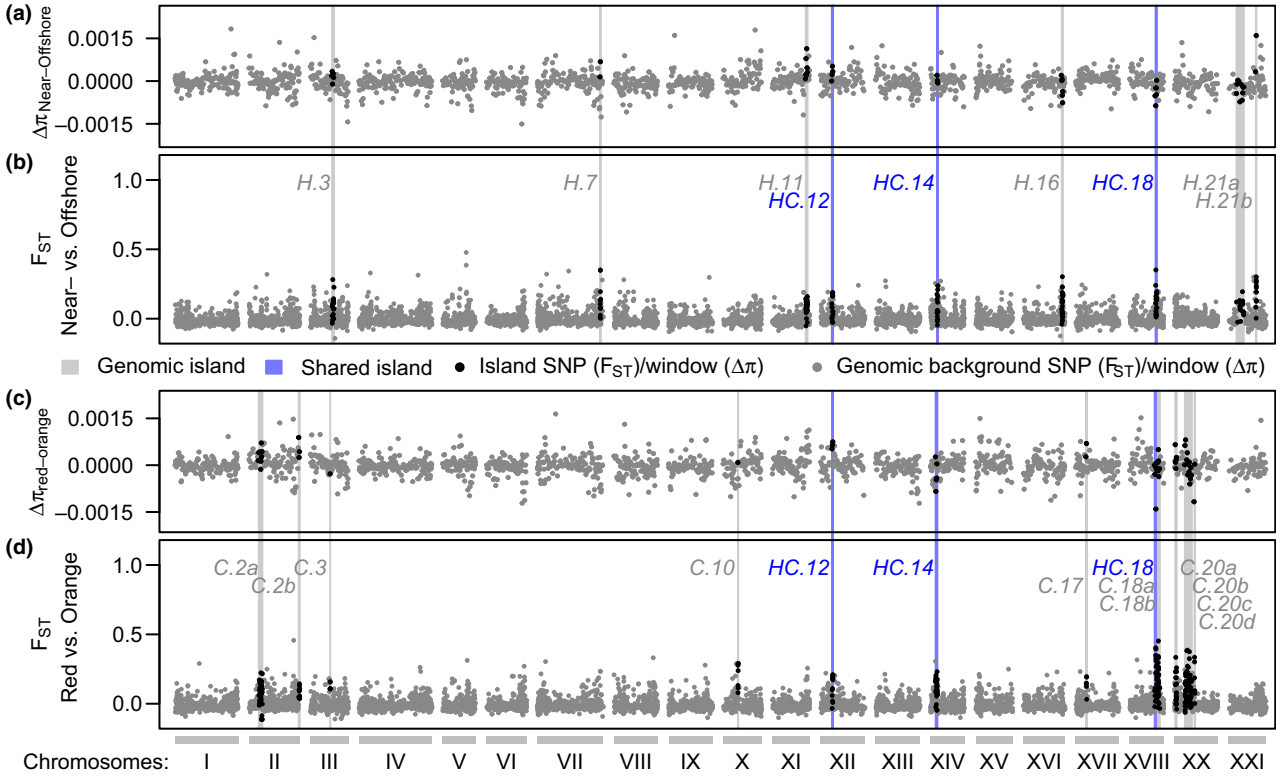

Fig. 5 Distribution of pairwise differentiation (F_{ST}) and differences in nucleotide diversity ($\Delta\pi$) across the genome between Jordeweiher males grouped by colour morph and breeding habitat. Genomic islands, regions with an accumulation of increased differentiation loci, are named with italic letters (see Table 1) and highlighted with grey vertical bars, black coloured SNPs (F_{ST}) and black coloured overlapping windows ($\Delta\pi$) respectively. Three genomic islands on chrXII, chrXIV and XVIII are found both among males grouped by colour morph and habitat (blue vertical bars). While on average, stickleback males are not differentiated across most of their genome, genomic islands harbour moderately divergent SNPs, ranging up to F_{ST} = 0.46 (colour morphs) and F_{ST} = 0.48 (habitat), respectively.

test, $P > 0.05$). Furthermore, none of these traits are differentiated among Jordeweiher males, while none of the few QTL known to influence male nuptial coloration overlap with the observed genomic islands (Malek *et al.* 2012; Yong *et al.* 2015). Unlike the analysis of candidate genes, the analysis of QTL overlap thus did not reveal plausible functional connections between divergent phenotypes and the genetic basis of traits detected in other studies and populations.

Most of the genomic islands that we found overlap with genomic islands previously reported between other stickleback ecotypes and populations (Table S2, Supporting information): islands C.2a, C.2b, H.3, H.11, HC.12, C.20b/c and H.21a are also differentiated between parapatric lake and stream ecotypes in Canada, Germany and Switzerland (Kaeuffer *et al.* 2012; Feulner *et al.* 2015; Marques *et al.* 2016). Islands C.20a/b/c and H.21a were also divergent between multiple parapatric marine and freshwater stickleback populations from around the Northern Hemisphere (Jones *et al.* 2012b). Finally, islands C.3, H.11 and C.20a contain loci divergent among allopatric marine and freshwater populations (DeFaveri *et al.* 2011) and loci with evidence for balancing selection in marine and freshwater populations were detected in islands C.2b, H.7 and C.20c/d (Makinen *et al.* 2008b). With the exception of sympatric lake and stream stickleback from Lake Constance, which also differ in red/orange throat coloration (Marques *et al.* 2016), most of these other cases involved differentiation between allopatric or parapatric populations, for which despite the obvious habitat differences, differences in male nuptial coloration have not been reported.

Discussion

Our results reveal a rare case in stickleback of strong differentiation in a sexual signalling phenotype associated with habitat differences in sympatry, in the absence of differentiation in ecological and morphological traits related to resource acquisition and predator defence. The genomic landscape associated with this early divergence is characterized by multiple genomic

Table 2 Position and size of genomic islands of differentiation among male colour morphs (C) and males breeding in different habitats (H), as well as islands found in both comparisons (HC)

Island name	Chromosome	Start*	End*	Length	No. of SNPs
H.3	chrIII	10 195 189	11 013 253	818 065	18
H.7	chrVII	29 369 008	29 580 946	211 939	12
H.11	chrXI	15 670 181	16 461 683	791 503	21
HC.12	chrXII	5 238 787	5 776 374	537 588	19
HC.14	chrXIV	2 990 195	3 400 864	410 670	18
H.16	chrXVI	17 953 430	18 437 334	483 905	26
HC.18	chrXVIII	12 115 653	12 700 449	584 797	21
H.21a	chrXXI	3 569 648	6 950 491	3 380 844	18
H.21b	chrXXI	12 637 551	12 876 396	238 846	10
C.2a	chrII	4 559 861	6 181 686	1 621 825	46
C.2b	chrII	23 256 982	23 687 419	430 437	11
C.3	chrIII	9 275 841	9 275 999	158	6
C.10	chrX	6 932 366	7 012 361	79 995	7
HC.12	chrXII	5 387 615	5 706 897	319 282	14
HC.14	chrXIV	2 377 579	3 131 078	753 499	33
C.17	chrXVII	4 900 840	5 033 507	132 667	6
HC.18	chrXVIII	11 702 348	12 700 449	998 101	28
C.18a	chrXVIII	13 194 103	13 453 530	259 427	15
C.18b	chrXVIII	13 483 346	14 067 086	583 740	10
C.20a	chrXX	363 978	956 341	592 363	21
C.20b	chrXX	4 850 891	6 519 852	1 668 961	44
C.20c	chrXX	6 619 982	8 049 063	1 429 081	21
C.20d	chrXX	9 363 001	9 607 463	244 462	6

*Coordinates from the reassembly by Glazer *et al.* (2015).

islands of moderate differentiation located on several chromosomes. In many islands, diversity is reduced in one of the two morphs but not the other one, suggesting that selective sweeps occurred in both morphs but at different loci. We identified a number of possible targets of divergent selection in genomic islands of differentiation, genes that are involved in visual perception and eye morphogenesis.

Environmentally mediated divergent sexual selection as a likely driver of stable throat colour polymorphism

Nuptial coloration is a product and target of sexual selection (Kodric-Brown & Brown 1984; Andersson 1994), with throat colour being of particular importance in threespine stickleback (Bakker & Mundwiler 1994; Rush *et al.* 2003; Flamarique *et al.* 2013). Previous work on other stickleback populations showed that males with redder throats are preferred by females (Bakker & Mundwiler 1994), more dominant (Bakker & Milinski 1993), more successful in defending territory and offspring (Candolin & Tukiainen 2015) and in a better condition (Milinski & Bakker 1990; Boughman 2007). However, sexual selection on throat colour has also been shown to be divergent between some populations and ecotypes, mainly depending on divergent visual environments (McKinnon & Rundle 2002). For example in stained waters on the North American Pacific coast, stickleback males have repeatedly acquired black throats (Semler 1971; Reimchen 1989; McKinnon 1995), a consequence of sexual selection maximizing male signal intensity or visibility to females against a background that is dominated by red light (Reimchen 1989; Boughman 2001; Lewandowski & Boughman 2008). Two studies (Malek *et al.* 2012; Yong *et al.* 2015) have identified a genetic basis for throat colour controlling hue (red vs. black) and intensity (redness), confirming a certain degree of heritability for this sexual signal. Theory suggests that interactions between sexual selection and visually heterogeneous habitats lead to the evolution and maintenance of male colour polymorphism under many conditions (Chunco *et al.* 2007) and many examples exist for environment-associated polymorphisms in male ornaments (Gray & McKinnon 2007) in guppies (Endler 1983; Cole & Endler 2015), cichlids (Seehausen & van Alphen 1999; Allender *et al.* 2003), killifish (Fuller 2002), silversides (Gray *et al.* 2008) or Anolis lizards (Leal & Fleishman 2002).

The strong and stable association between male colour morph and breeding habitat in the Jordeweiher pond is likely driven by such environment-dependent divergent sexual selection. In another study (Feller *et al.* 2016), we found a bimodal distribution of female preferences in this population indicating that the female

population in this pond does not cause directional selection towards redder throat coloration. Instead, females vary in their preferences for either red or orange males even when tested in the same standard white light laboratory environment, suggesting that some level of assortative mating could be present in the pond (Feller *et al.* 2016). Red and orange nuptial coloration could therefore be alternative strategies to maximize male attractiveness to females in different light regimes and against different background colours, in response to divergent sexual selection imposed by females. Divergence in nest types as an extended phenotype (Hunter 2009) may enhance male attractiveness in the respective habitats (Kraak *et al.* 1999; Bolnick *et al.* 2015): nearshore males build shallower, less conspicuous, hidden nests (Fig. 1b), while offshore males build open, crater-shaped nests at greater depth (Fig. 1e). Both direct sexual selection against males in the 'wrong' habitat, male–male competition and 'habitat-matching' (Edelaar *et al.* 2008), the active choice of the optimal breeding habitat maximizing the impact of a male morph's sexual signalling phenotype, may contribute to the stability of this polymorphism.

Divergence in throat colour could also be a product of the interaction between disruptive natural and sexual selection between the two habitats: Predators may select for reduced conspicuousness and camouflage, leading to different solutions in the two light regimes and background colours. This could induce a trade-off between natural and sexual selection, which in turn may have caused offshore males to compensate for being less red-throated by building more elaborate nests that might aid in attracting females as shown elsewhere (Kraak *et al.* 1999). Also, predator composition and predation pressure may vary between habitats. However, the predator fauna of the Jordeweiher is very impoverished compared with other stickleback habitats (Zeller *et al.* 2012), in particular piscivorous fish and birds – the latter putatively causing divergent predation pressure between habitats – are rare and divergent selection imposed by these predators may thus be erratic and overall not very strong. Furthermore, the magnitude of trait divergence was much larger in throat coloration than in typical predator defence- or predator evasion-related traits (e.g. swimming performance), which was unexpected if predator composition or predator pressure differences between habitats would be a major source of divergent natural selection.

Little divergence in traits under direct natural selection

Traits commonly found to be under direct natural selection, such as predator defence, feeding ecology or swimming performance traits, had not diverged between habitats or colour morphs in the Jordeweiher. This is in strong contrast to most other cases of phenotypic divergence between stickleback populations occupying adjacent habitats, which commonly show strong morphological divergence in traits related to predator avoidance or feeding, rather than, or simultaneously with, divergence in sexually selected traits (McPhail 1994; McKinnon & Rundle 2002; Olafsdottir *et al.* 2006, 2007a,b; Cooper *et al.* 2011; Ravinet *et al.* 2013; Reimchen *et al.* 2013). Most well-studied stickleback ecotypes with divergence in mating signals show morphological divergence related to feeding and/or predator defence too, for example sympatric benthic and limnetic stickleback species in British Columbia (Schluter & McPhail 1992; McPhail 1994; Boughman *et al.* 2005), sympatric lake and stream stickleback from Lake Constance (Lucek *et al.* 2012; Moser *et al.* 2012) or allopatric stickleback from stained vs. clear lakes on Haida Gwaii (Reimchen *et al.* 2013).

While a range of differences in habitats and selection regimes may explain phenotypic divergence between allopatric or parapatric populations, the major axis of phenotypic divergence in stickleback species coexisting in sympatry is benthic vs. limnetic forms in freshwater lakes in British Columbia (McPhail 1994). Although these forms are thought to have evolved from double invasions of the lakes rather than from sympatric speciation (Taylor & McPhail 2000), ecological differentiation in sympatry is likely crucial to their coexistence and persistence (Schluter & McPhail 1992; Rundle *et al.* 2000; Vamosi & Schluter 2002; Arnegard *et al.* 2014). The weak divergence in ecological traits between habitats and colour morphs in the Jordeweiher pond despite strong differentiation in mating traits may suggest that the fitness landscape for feeding-related traits in this habitat does not cause strong disruptive selection, contrary to benthic and limnetic stickleback in Canadian lakes (Arnegard *et al.* 2014). The different predator community in the Jordeweiher, dominated by insects, adds to generating a selective landscape that is probably very different from those of the British Columbia lakes where trout as a predator is important (Vamosi & Schluter 2002; Rundle *et al.* 2003; Arnegard *et al.* 2014). Alternatively, it is possible that disruptive selection in Jordeweiher is dissipated by ecological dimorphism between the sexes instead of divergent ecological adaptation between colour morphs (Bolnick & Lau 2008; Bolnick 2011; Cooper *et al.* 2011).

Genomic signature of early ecological speciation

While sympatric benthic and limnetic stickleback species from lakes in British Columbia show considerable

reproductive isolation and genomic differentiation (McPhail 1994; Nagel & Schluter 1998; Rundle *et al.* 2000; Boughman 2001; Jones *et al.* 2012a), genome differentiation among Jordeweiher ecotypes is restricted to a few genomic islands of significantly elevated differentiation, similar to sympatric lake and stream ecotypes from Lake Constance (Marques *et al.* 2016). The evolution of Canadian benthic and limnetic stickleback species pairs involved an extensive phase of allopatry (Taylor & McPhail 2000) and genomic differentiation may reflect a mix of selective maintenance of adaptive differentiation, adaptive divergence in sympatry and random divergence due to historical contingency (Jones *et al.* 2012a). The Jordeweiher pond instead, as most of the surrounding populations in Central Switzerland, is inhabited by a population that arose from hybridization between at least two distinct stickleback lineages (Lucek *et al.* 2010; Roy *et al.* 2015) and the resulting genetic and phenotypic variation in the hybrid swarm may have facilitated incipient speciation into colour morphs divergently adapted to two adjacent habitat and therein 'ecotypes'. The fact that we find no elevated background differentiation in the genome with a number of genomic islands is consistent with the hypothesis that the Jordeweiher pond was colonized only once by a population from the hybrid zone rather than separately by each of the different lineages that gave rise to the hybrid zone. It is therefore likely that genomic differentiation and stabilization among Jordeweiher nearshore and offshore ecotypes is a product of very recent incipient speciation in sympatry, possibly facilitated by the preceding formation of a hybrid swarm between divergent lineages (Seehausen 2004, 2013).

Few well-documented examples of sympatric divergence exist (Bolnick & Fitzpatrick 2007), and genomic differentiation has been studied in even fewer cases. Of the two cases that we know of, *Rhagoletis* fruit flies and crater lake cichlids (Michel *et al.* 2010; Malinsky *et al.* 2015), many genomic islands have been found, similar to the Jordeweiher stickleback. However, in *Rhagoletis* fruit flies many of these islands were associated with inversions that diverged during periods of allopatry, something that remains unknown in Jordeweiher stickleback and the crater lake cichlids. In contrast to the Jordeweiher stickleback, weak but significant genomewide background differentiation was detectable in fruit flies diverging for 150 (Michel *et al.* 2010) and cichlids diverging for 10 000 years (Malinsky *et al.* 2015). These differences in genomic background differentiation might be due to a combination of variation in time since divergence started, levels of ongoing gene flow, and the mechanisms of reproductive isolation, and varying population sizes and thus drift in different systems.

What are the traits coded in genomic islands under divergent selection? The presence of multiple moderately differentiated islands in Jordeweiher stickleback suggests a rather complex genetic basis for the traits under selection, controlled by genes on different chromosomes, and/or multifarious selection on several traits leading to multiple differentiated genomic regions (Feder *et al.* 2012). The presence of colour perception and eye development genes may indicate that the perception of colour and therefore female preferences are targets of divergent sexual selection (Fig. 5). If female preference was environment-dependent and genetically inherited, reproductive isolation between ecotypes could be strengthened by sensory drive, the combination of habitat-specific transmission of male signal, perception adaptation in females and the matching of male signal and female perception (Boughman 2002). Sensory drive speciation is well known from benthic and limnetic stickleback (Boughman 2001) and from *Pundamilia* cichlids (Seehausen *et al.* 2008) and may have led to sympatric speciation in the latter (Seehausen & van Alphen 1999; Seehausen *et al.* 2008). We do however not yet know whether sensory drive may operate as a mechanism of divergence among Jordeweiher sticklebacks. Measurement of the distribution of female mate preferences, excluding environmental effects, revealed a bimodal preference function among females (Feller *et al.* 2016), yet the strength of assortative mating under natural conditions remains unknown (Snowberg & Bolnick 2012). A better understanding of the environmental component of mate choice will be crucial to evaluate whether sensory drive may be operating and causing reproductive isolation in the Jordeweiher stickleback (Hendry *et al.* 2009).

Conclusions

We showed that two sympatric colour morphs of threespine stickleback with a stable habitat association evolved in a 90-year-old population, representing a very early stage of ecological speciation as defined by the emergence of divergence in multiple genomic regions in sympatry. The Jordeweiher pond stickleback are the youngest case of divergence between sympatric colour morphs investigated at the genomic level, and thus, the first snapshot of the genomic landscape associated with very early ecological speciation in which divergent sexual selection likely plays the lead role. Our results suggest that the genomic pattern associated with this process is characterized by multiple unlinked genomic islands against an undifferentiated genomic background. We encourage further search for other young sympatric colour polymorphisms in stickleback, the genomic investigation of which would allow testing the generality of this pattern.

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D.A.M., O.S., K.L., M.P.H. and A.F.F. collected data in the field, D.A.M., K.L. and A.F.F. analysed the data with assistance from L.E. and O.S., D.A.M. wrote the manuscript, and O.S., K.L., L.E., J.I.M. and C.E.W. revised the manuscript.

Data accessibility

FASTQ files with demultiplexed and base quality score recalibrated reads have been deposited in the short read archive (www.ncbi.nlm.nih.gov/sra) under Accession no. SRP079408, ecological and morphological data on Dryad (doi: http://dx.doi.org/10.5061/dryad.js08q).

Supporting information

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

Fig. S1 Linear and geometric morphometric traits measured in this study.

Fig. S2 Variation in linear morphometric traits among males grouped by habitat and throat colour.

Fig. S3 Variation in body shape among males grouped by habitat and throat colour, showing a trend for shorter heads and more slender bodies in offshore/orange males.

Fig. S4 Stomach content and stable isotope data for males from 2007.

Table S1 Candidate genes in genomic islands with functions relevant to phenotypic differentiation among Jordeweiher stickleback males.

Table S2 Table of QTLs and outlier regions identified in previous studies overlapping with genomic islands from this study.