globally for N_2 fixation[15]. Dust deposition at this location is highly episodic, and has varied widely on geological timescales[28]. If dust deposition can to some extent relieve both P and Fe limitation of diazotrophy, the postulated link between climate-driven changes in dust deposition and N_2 fixation may be even stronger then initially suggested[3,29]. □

Methods

Trace-metal clean techniques were strictly used throughout the preparation and execution of the experiments as this is crucial to the good survival of diazotrophs and for *Trichodesmium* spp. in particular[27]. Surface sea water was collected (1–3 m) after dark using a trace-metal clean diaphragm pump. Sea water was pumped into 60-l carboys from which it was siphoned into 1.18 l acid-washed polycarbonate bottles. Under a laminar flow hood, nutrients were added alone and in combination to final concentrations of 1.0 μM NH_4^+ + 1.0 μM NO_3^-, 0.2 μM NaH_2PO_4, and 2.0 nM $FeCl_3$. Saharan dust treatments were also conducted with final concentrations of 0.5 mg l^{-1} (D1) and 2 mg l^{-1} (D2). These concentrations were chosen to simulate concentrations in the upper 1 m of the water column after a strong Saharan aerosol deposition event[11]. The dust consisted of the fine fractions of surface soils collected in the Hoggar region (Southern Algeria) with a grain-size distribution and chemical composition typical for Saharan aerosols collected far from the source[10,11]. The measured P and Fe content of the dust was 0.14 ± 0.01% and 4.97 ± 0.49% (± one standard error). The phosphate liberated from the dust treatments was approximately 2.7 and 10.8 nmol l^{-1}, and Fe released was 0.9 and 3.6 nmol l^{-1}, for the 0.5 and 2.0 mg l^{-1} dust treatments respectively (see Supplementary Information). The bottles were then sealed gas tight and placed in an on-deck incubator with circulating surface sea water. Light was attenuated to 20% of incident surface values with blue filters (Lagoon Blue, Lee Filters #172). For each treatment, parallel incubations for each variable (carbon fixation, nitrogen fixation and biomass) were run in triplicate over 48 h with rate measurements made during the final 24 h and chlorophyll concentration determined at 48 h. At each of the three study sites, over 100 bottles were incubated. For measuring net nitrogen fixation rates, 1.0 ml of 99% $^{15}N_2$ was introduced to each bottle through a butyl septum using a gas-tight syringe. $^{15}N_2$ uptake measurements may underestimate nitrogen fixation if significant release of dissolved nitrogen occurs[30]. However, this effect should be minimal in our experiments because, in a N-limited oligotrophic system, our 24-h rate measurements should allow released labile dissolved N to be reincorporated into particulate matter. For measuring primary productivity, 0.1 mCi ^{14}C-bicarbonate was added to each bottle. All incubations were conducted from dawn-to-dawn and stopped by gentle filtration.

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Evidence for ecology's role in speciation

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A principal challenge in testing the role of natural selection in speciation is to connect the build-up of reproductive isolation between populations to divergence of ecologically important traits[1,2]. Demonstrations of 'parallel speciation', or assortative mating by selective environment, link ecology and isolation[3–5], but the phenotypic traits mediating isolation have not been confirmed. Here we show that the parallel build-up of mating

incompatibilities between stickleback populations can be largely accounted for by assortative mating based on one trait, body size, which evolves predictably according to environment. In addition to documenting the influence of body size on reproductive isolation for stickleback populations spread across the Northern Hemisphere, we have confirmed its importance through a new experimental manipulation. Together, these results suggest that speciation may arise largely as a by-product of ecological differences and divergent selection on a small number of phenotypic traits.

Anadromous and stream-resident threespine stickleback (*Gasterosteus aculeatus*) populations that presently breed sympatrically often show little introgression and exhibit pre-mating isolation in the laboratory[6,7] (but see ref. 8). Stream populations from different regions are phenotypically similar and the stream ecotype has evolved repeatedly, whereas the ancestral, anadromous ecotype has persisted in similar but geographically widespread marine habitats[6,9]. The repeated origin of like stream forms and the maintenance of the large anadromous phenotype in distant but similar habitats suggest that certain morphological traits, including body size, are principally adaptations to environmental selection regime[6,10], a view supported by experimental studies of other stickleback systems[6,7,11,12]. The large size of anadromous sticklebacks may be an adaptation to relatively long migrations[13] but other selective factors such as predation regime and food supply might also contribute. Differences in size between stickleback populations have been shown to be substantially heritable in common-garden studies[7,14], including one of anadromous and stream populations from California[13] and a recent investigation that identifies a major quantitative trait locus for size distinguishing a Japanese marine and a British Columbia freshwater population[15].

The wide distribution of stream and anadromous sticklebacks provides exceptional replication for investigation of the factors causing reproductive isolation[6,7]. We collected subjects for mating trials from geographically distant regions (Alaska, British Columbia, Iceland (stream only), Scotland, Norway (stream only) and Japan; details in Supplementary Information) chosen to maximize the probability that the stream populations had evolved independently and that the anadromous populations were physically isolated from one another, with minimal gene flow between them. Thus we focused primarily on sites that had been recently glaciated and were separated from other study locales by large expanses of land, sea or both. Nested AMOVA's (analysis of molecular variance) based on microsatellite data support our assumption of close relationships among geographically adjacent populations rather than those of the same ecotype (Table 1). These analyses consistently reveal that much of the variation in microsatellites is accounted for by geography, as expected if stream populations have evolved repeatedly from anadromous populations inhabiting the same region. Ecotype should account for a significant proportion of molecular variance if the stream ecotype evolved just once then spread to the various study sites, but this is never observed. Results from analyses of allozymes[16] and mitochondrial DNA sequences[17] also suggest replicated origins of freshwater populations from anadromous or marine ancestors, and distant relationships between far-flung anadromous populations. Introgression within regions and insufficient time for full lineage sorting may contribute to these patterns; however, the glacial history of most of our study areas and the well documented rapid pace of stickleback evolution[18] support the hypothesis that the stream-resident ecotype has evolved repeatedly.

Consistent with earlier reports[6,10], anadromous populations in our study possessed larger average body sizes than the stream populations (mean anadromous female length = 69.2 mm, standard error (SE) = 5.3 mm; mean stream female standard length = 46.6 mm, SE = 1.6 mm; $P = 0.001$, two-tailed t-test, $n = 10$ populations; a paired test for regions with both ecotypes was also significant). To assess reproductive compatibility or isolation, recently collected fish from all sites were brought together in the laboratory for mating tests.

Nearby stream and anadromous pairs exhibited overall high, significant isolation. Courtship was more than twice as successful between pairs of the same ecotype, and in this case from the same population, relative to pairs composed of different ecotype individuals (Fig. 1; see also refs 19, 20).

A significant pattern of parallel speciation, more precisely parallel reproductive isolation, emerged in tests of pairs composed exclusively of individuals from different regions (Fig. 1). Despite independent origins and/or great distances between populations, female stream and anadromous sticklebacks each preferred males of their own ecotype. This pattern provides a strong signature of divergent

Table 1 **Results of AMOVA**

Grouping model	Percentage of variation	F_{ST}/ϕ_{ST}
Continent (all populations)		
Among continents	7.85/20.90	0.08‡/0.21‡
Among populations in continent	14.93/17.54	0.16‡/0.22‡
Within populations	77.22/61.56	0.23‡/0.38‡
Ocean (all populations)		
Atlantic versus Pacific	6.13/13.35	0.06†/0.13†
Among populations in ocean	17.13/25.23	0.18‡/0.29‡
Within populations	76.74/61.42	0.23‡/0.39‡
Ecotype (all populations)		
Anadromous versus stream	−0.07/−0.91	−0.01/−0.01
Among populations in ecotype	21.04 / 35.13	0.21‡/0.34‡
Within populations	79.04/65.79	0.21‡/0.34‡
Region (single ecotype regions excluded)		
Among regions	2.32/12.12	0.02*/0.12†
Among populations in regions	19.65/26.20	0.20‡/0.29‡
Within populations	78.03/61.69	0.21‡/0.38‡
Ecotype (single ecotype regions excluded)		
Anadromous versus stream	−0.80/−2.61	−0.01/−0.01
Among populations in ecotype	22.24/39.13	0.22‡/0.38‡
Within populations	78.56/63.48	0.21‡/0.36‡

Values to the left of the solidus represent F_{ST}-based analyses whereas values to the right of the solidus represent ϕ_{ST}-based analyses (all conducted with Arlequin, as were permutation tests of significance). The former incorporates allele frequency variation only; the latter incorporates variation in allele size and frequency. Continents are Europe (Iceland, Norway, Scotland), Japan (that is, Asia) and North America (Alaska, British Columbia). Regions are defined as groups of populations from Alaska, British Columbia, Iceland, Japan, Norway and Scotland, but only regions for which data are available both from anadromous and stream-resident populations are included in these analyses of region; thus Norway and Iceland stream populations are excluded. Estimates of divergence time between populations, based on $\delta\mu^2$, range from less than 5,000 to over 200,000 years and are given in Supplementary Information along with additional phylogenetic analyses and tables of genetic distances.
*$P < 0.1$.
†$P < 0.01$.
‡$P < 0.001$.

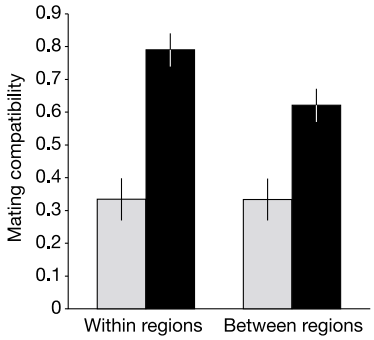

Figure 1 Mating compatibility (mean proportion of trials involving a nest inspection) for same (black) and different ecotype (grey) combinations, for within region tests ($P = 0.0058$, paired one-tailed t-test, $n = 7$ populations) and between region tests ($P = 0.0045$, $n = 9$; when the genetic distance measure $\delta\mu^2$ and ecotype match were analysed together, only ecotype was significant: $\delta\mu^2$, $P = 0.083$; ecotype match, $P = 0.0036$, one-tailed t-tests, $n = 9$). Total $N = 850$ (see Supplementary Information). Error bars are 1 SE.

selection[3], suggesting that reproductive isolation is brought about by adaptation to different environments. Moreover, these patterns stand out at vast distances, showing that the influence of ecology and selection is sufficient to be detectable even above the background variation that must exist on such a scale.

Levels of reproductive isolation are well accounted for by differences in a single trait, body size. Stream sticklebacks are typically smaller than anadromous fish and the probability of mating between populations is negatively related to size difference, even after accounting for the time that populations have been separated (Fig. 2). When body size difference and ecotype match are considered together, only size difference is significant ($P = 0.014$; for ecotype, $P = 0.10$, one-tailed t-tests, $n = 9$ in both cases). Thus size-assortative mating seems to be a very general tendency, with mating preferences and patterns that cause reproductive isolation changing largely as by-products of differences in body size. Nevertheless, the near significance of the term for residual ecotype match raises the possibility that traits other than body size make a secondary contribution.

Experimental manipulation of body size directly confirms the connection between size divergence and the build-up of reproductive isolation. We raised large and small females of each ecotype from both Japan and British Columbia, mainly by providing long and short growing periods, and tested mating patterns using field-collected British Columbia males. As predicted, the preferred male ecotype depended on the size to which the female was raised (Fig. 3). Usually isolated, different ecotype combinations were compatible if size differences were small as a result of the manipulation; for example, experimentally induced small British Columbia anadromous females and naturally small field-caught stream males courted with considerable success (mean standard length difference = 3.5 mm, mating compatibility = 0.5). Conversely, same-ecotype pairings were relatively unsuccessful when manipulation led to large size differences; for example, mating compatibility was just 0.25 for experimentally induced small British Columbia anadromous females with large field-caught anadromous males (mean standard length difference = 17.5 mm, Fig. 3). This mating pattern did not vary significantly with female ecotype or region. Females also retained a statistically independent and significant, albeit weaker, preference for males of their own ecotype (Fig. 3), once again suggesting that traits other than size do make a secondary contribution to reproductive isolation.

Many of the populations in our comparative analyses are completely allopatric to one another, suggesting that interactions in sympatry, such as through reinforcement[21,22], are not essential for the evolution of reproductive isolation. Indeed, populations sympatric with the opposite ecotype did not show stronger preferences for their own population than did allopatric populations not sympatric with the opposite ecotype (same ecotype – different ecotype mean mating compatibility, sympatric populations versus allopatric: $P = 0.433$, one-tailed t-test, $n = 6$), although there was considerable variation in levels of isolation among pairs[8]. Similar results, in terms of preference for the same ecotype, were obtained in the between-region tests for populations that do or do not co-occur with the opposite ecotype ($P = 0.424$, one-tailed t-test, $n = 9$). The pattern of reproductive character displacement may not be observed here, in contrast with some other studies of sticklebacks[6,21], because it is masked by the large size differences in many allopatric comparisons and because we did not conduct paired tests of sympatric and allopatric populations within lineages, which is a more sensitive assay.

In contrast to body size, divergence in red colouration does not appear to be an important cause of reproductive isolation in the stream–anadromous stickleback system. Interpopulation assortative mating based on male colouration was not significant for these populations (for allopatric population combinations, $P = 0.76$, one-tailed t-test, $n = 9$ populations). This finding may differ from results for lake populations because of less extreme colour differences in this system than between lake sticklebacks[23].

Our comparative analyses and experimental manipulation together demonstrate the importance of a single phenotypic trait, body size, in stickleback speciation and suggest that divergent selection on this trait makes a predominant contribution to reproductive isolation. Although size has previously been shown to be important in isolation between pairs of stickleback populations[1,24], it has not been shown to account for patterns, including parallel

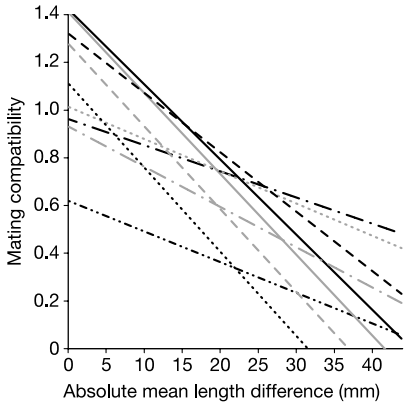

Figure 2 Regression lines for mating compatibility (arcsine square-root-transformed) on absolute mean standard length difference for each female population (Supplementary Information) tested with allopatric male populations. The decline of mating compatibility with increasing difference in body size is highly significant ($P < 0.0001$, one-tailed t-test, $n = 9$). The relationship between genetic distance/time ($\delta\mu^2$) and reproductive isolation was not significant ($P = 0.10$) and remained so when analysed with size difference ($P = 0.070$), whereas size continued to be significant ($P < 0.0001$). Statistical significance of these results is unchanged in Mantel tests. Regressions of mating compatibility on Nei's standard distance and (1-proportion shared alleles) were each just significant ($P = 0.035$ and 0.018, respectively) but did not approach significance in joint analyses with size difference ($P > 0.13$ in both cases).

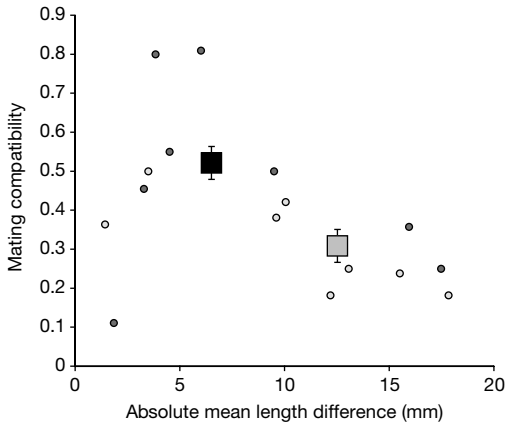

Figure 3 Mating compatibility versus absolute mean standard length difference between males and females, with female size manipulated. Circles represent each combination of female region, ecotype, size manipulation and male ecotype, with same ecotype pairings coloured dark grey. Squares are mean values for pairs manipulated to be similar (black: large (size-manipulated) females with anadromous males, which are large, and small females with stream males, which are small) or different (grey: large females with stream males and small females with anadromous males) in size ($N = 262$; error bars are 1 SE). The preferred male ecotype depended on the size to which females were manipulated ($\chi^2 = 13.40$, $P = 0.0003$, degrees of freedom = 1, 251) although females also retained a preference for males of their own ecotype ($\chi^2 = 7.46$, $P = 0.0063$, degrees of freedom = 1, 251).

isolation, among sets of populations; nor has its importance been confirmed previously through a manipulation. The comparative patterns documented here were obtained with populations separated, in some cases, for hundreds of thousands of years, extending the role of divergent selection and ecology well beyond that implied by previous work[4,5].

The malleability of body size raises the possibility that multiple mechanisms underlie reproductive isolation between populations differing in size; this topic deserves further investigation. In addition to directly heritable effects, phenotypic plasticity may contribute to the large size of anadromous fish through rapid growth in the relatively productive temperate marine habitat. In the extreme case, this mechanism might provide an alternative explanation for our results, if it were the main cause of size differences between some stream and anadromous populations. But whether directly heritable or a plastic by-product of evolved migratory differences and habitat choice, stream–anadromous size differences are essentially ecological in origin and can yield reproductive isolation only when coupled with a widespread tendency towards size-assortative mating. □

Methods

Collection and maintenance of fish

Sticklebacks were collected in the spring of each year using minnow traps and hand seines. They were transported to Vancouver (1996) or Whitewater (2000, 2001) and maintained using standard procedures[25].

Microsatellites and genetic distances

Microsatellites were genotyped[26] and used to calculate $\delta\mu^2$, the appropriate genetic distance measure when timeframes allow for mutation. We excluded loci violating the stepwise mutation assumption, leaving 15 microsatellites from 22 fish per population. We conducted supplemental analyses using two other genetic distances.

Mating trials

Protocols in 30-min 'no choice' trials, conducted with a nesting male and a single female in a 96-l aquarium, were essentially identical to those described in previous studies[1,4]. Before a male's first trial (2000 and 2001), we scored his red colouration rank visually[23].

We used nest inspection as our principal measure of mating compatibility because females from some populations exhibited very low levels of nest entry with all male populations. Furthermore, nest inspection may be a more robust measure of preference than nest entry, which is more influenced by changes in female state[27].

To ensure statistical independence of observations, we used female population as the unit of replication in most analyses, following ref. 4. Tests of assortative mating used paired *t*-tests to compare average frequency of female nest inspection with males of 'same ecotype' and 'other ecotype' (ecotype match). In tests of assortative mating within a region, 'same ecotype' refers to males from the female's own population, whereas 'other ecotype' refers to males from the adjacent population but of the opposite life history. In the main, conservative test of parallel reproductive isolation, 'own ecotype' refers to males with the same life history as females but from other regions, whereas 'other ecotype' includes all males from other regions and with the opposite life history (following refs. 3, 4).

Effects of body size, genetic distance and colouration on mating compatibility were tested with one-sample *t*-tests. The single measurement for each female population was the slope of a linear regression of nest inspection frequency against genetic distance from each male population, or against absolute difference in body size or male colouration, weighting by sample size. The latter analyses excluded data for sympatric populations to avoid any direct influence of familiarity or coevolution. Multiple regression analyses were used to assess independent variables simultaneously, and mean slopes again compared. Ecological similarity, critical to tests of parallel isolation, was included in some analyses using the dummy variable 'ecotype match'. The arcsine transformation was applied to proportions. Standard and partial Mantel tests were used to check regression results using NTSYSpc V 2.11. We focus on regression results owing to possible problems with partial Mantel tests[28] but our main findings are statistically robust.

In the central analyses of parallel reproductive isolation, the observed distribution of genetic distances accounts less well for mating patterns, in terms of shared evolutionary history, than a star phylogeny possessing no evolutionary structure (unrooted tree with all internal branches set to 0, tip branches to 1 (ref. 29); shared evolutionary history was calculated from a tree based on $\delta\mu^2$ and constructed using PHYLIP's 'Kitsch' v. 3.6a3; best log-likelihood for star phylogeny = 6.32, for Fitch–Margoliash = 4.26). Consequently, we have made no additional statistical adjustments for shared history beyond the incorporation of $\delta\mu^2$ into some analyses.

Size manipulations

Crosses were made and sticklebacks initially raised using standard procedures[30]. At approximately 8 weeks of age, all juvenile sticklebacks were culled to a maximum density of 45 individuals per 96-l aquarium. Fish were raised at these high densities to one year of age to produce a small body size. Those fish destined to be raised to two years of age and large size were further reduced to a maximum density of 25 fish per aquarium for their second year; densities were sometimes lower and variable, however, due mainly to mortality. Fifty separate crosses were made and an average of 5.2 females tested from each cross for a total of 262 trials, all conducted with wild-caught British Columbia stream and anadromous males.

We analysed the resulting data with a logistic regression on nest inspection that included the effects female size treatment, female region, female ecotype, male ecotype and their interactions. The key experimental prediction was that female sticklebacks manipulated to large size would be relatively more compatible with anadromous males, which are large, whereas fish manipulated to small size would be more compatible with stream males, which are small. Statistically, this should be manifest in a significant interaction between female size treatment and male ecotype. Assortative mating by ecotype would contribute to a significant male ecotype by female ecotype interaction term. Three-way interactions and the four-way interaction were nonsignificant and omitted from the main analysis. Additional details, including the combinations of populations tested in the comparative experiment, are given in Supplementary Information. Experiments were approved by the UW-Whitewater animal care committee (IACUC).

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Zinc transporter LIVI controls epithelial-mesenchymal transition in zebrafish gastrula organizer

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Vertebrate gastrulation is a critical step in the establishment of body plan. During gastrulation, epithelial-mesenchymal transition (EMT) occurs[1]. EMT is one of the central events of embryonic development, organ and tissue regeneration, and cancer metastasis[1,2]. Signal transducers and activators of transcription (STATs) mediate biological actions such as cell proliferation, differentiation and survival in response to cytokines and growth factors, in a variety of biological processes[3–6]. STATs are also important in EMT during gastrulation, organogenesis, wound healing and cancer progression[7–9]. We previously showed that STAT3 is activated in the organizer during zebrafish gastrulation and its activity is essential for gastrulation movements. The requirement for STAT3 is cell-autonomous for the anterior migration of gastrula organizer cells, and non-cell-autonomous for the convergence of neighbouring cells[10]. The molecular mechanisms of STAT's action in EMT, however, are unknown. Here we identify LIV1, a breast-cancer-associated zinc transporter protein[11–13], as a downstream target of STAT3 that is essential and sufficient for STAT3's cell-autonomous role in the EMT of zebrafish gastrula organizer cells. Furthermore, we demonstrate that LIV1 is essential for the nuclear localization of zinc-finger protein Snail, a master regulator of EMT[1,2,14,15]. These results establish a molecular link between STAT3, LIV1 and Snail in EMT.

We isolated a complementary DNA encoding zebrafish *LIV1* (*LZT-Dr3*; Supplementary Fig. 1a) by a subtraction screening. LIV1 belongs to a subfamily of ZIP zinc transporters (Zrt-, Irt-like proteins), termed LZT (LIV-1 subfamily of ZIP zinc transporters)[16]. Its sequence contains eight transmembrane domains (TM) with a long extracellular amino terminus, a short extracellular carboxy terminus, a long variable region in the cytoplasmic loop between TM III and IV, an HNF motif in TM IV, a HEXPHE motif in TM V, and numerous histidine-rich repeats. All these motifs indicate LIV1's relation to the LZT subfamily of ZIP zinc transporters[16]. The phylogenetic tree and alignment across the transmembrane domains IV and V of the LZT subfamily of ZIP zinc transporters revealed that the cDNA was the zebrafish counterpart of human *LIV1* (*LZT-Hs3*; Supplementary Fig. 1b). The zebrafish *LIV1* messenger RNA was expressed maternally, and zygotic transcripts increased at the shield stage and accumulated in the prechordal mesendoderm cells, in which STAT3 is activated[10] (Fig. 1a–f). The expression of *LIV1* mRNA in the gastrula organizer was abolished in STAT3-depleted embryos, indicating that *LIV1* is a downstream target of STAT3 (Fig. 1g, h). Consistent with this, gp130-stimulation by G-CSF induced *LIV1* expression in the mouse proB cell line, Baf/B03 cells expressing a chimaeric receptor G-CSFR-gp130[17] (Fig. 1i, G133), but not the Baf/B03 cells expressing the mutated chimaeric receptor defective in STAT3 activation (Fig. 1i, G133F3). Furthermore, the dominant negative form of STAT3 inhibited gp130-meditated *LIV1* expression in Baf/B03 cells (Fig. 1i, G133-STAT3F). Moreover, transfection of siRNA for human *STAT3* in the human prostate cell line, DU145 cells downregulated the expression of human *LIV1* (Fig. 1j). Together, these results showed that the *LIV1* gene is regulated by STAT3 not only in zebrafish organizer cells but also in some mammalian cells.

To address the early embryonic roles of LIV1 during zebrafish gastrulation, we analysed embryos lacking LIV1 activity using an antisense morpholino[18] (LIV1-MO). Embryos receiving injections of LIV1-MO displayed a mispositioned head and shortened anterior–posterior axis at the end of gastrulation and later stages, whereas the injection of control LIV1D4-MO did not lead to any obvious phenotype (Fig. 2a–d). The axial hypoblast layer (notochord and somite) was formed in LIV1-MO-injected zebrafish embryos, but was shortened and thickened, suggesting that anterior movement of the axial mesendoderm was severely impaired, whereas the internalization, epiboly, and dorsal convergence move-

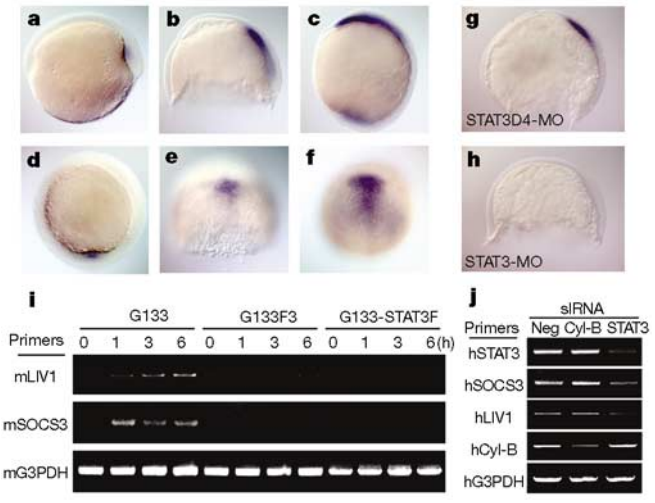

Figure 1 Zinc transporter LIV1 is a target of STAT3 in zebrafish gastrula organizer cells. Zebrafish *LIV1* cDNA was isolated by a subtraction screening using normal embryos and STAT3-depleted embryos. **a**–**h**, *LIV1* mRNA at gastrula stage in normal embryo (**a**–**f**), STAT3D4 control morpholino-injected embryo (**g**), and STAT3 morpholino-injected embryo (**h**). **a**–**c**, **g**, **h**, Lateral views, with dorsal to the right; **d**, **f**, Animal-pole views, with dorsal to the bottom; **e**, dorsal views. **i**, Baf/B03 transfectant cells expressing the chimaeric receptor (G133), mutated chimaeric receptor defective in STAT3 activation (G133F3), or both the receptor and dominant negative STAT3 (G133-STAT3F) were cultured with G-CSF for the indicated times. Total RNA was isolated and subjected to RT–PCR with LIV1, SOCS3 and G3PDH primers. **j**, DU145 cells were transfected with siRNA for either STAT3, negative control (Neg), or cyclophilin B (Cyl-B), followed by isolation of total RNA that was subjected to RT–PCR with primers STAT3, SOCS3, LIV1, cyclophilin B and G3PDH.