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GENE FLOW AND SELECTION ON PHENOTYPIC PLASTICITY IN AN ISLAND SYSTEM OF *RANA TEMPORARIA*

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Gene flow is often considered to be one of the main factors that constrains local adaptation in a heterogeneous environment. However, gene flow may also lead to the evolution of phenotypic plasticity. We investigated the effect of gene flow on local adaptation and phenotypic plasticity in development time in island populations of the common frog *Rana temporaria* which breed in pools that differ in drying regimes. This was done by investigating associations between traits (measured in a common garden experiment) and selective factors (pool drying regimes and gene flow from other populations inhabiting different environments) by regression analyses and by comparing pairwise F_{ST} values (obtained from microsatellite analyses) with pairwise Q_{ST} values. We found that the degree of phenotypic plasticity was positively correlated with gene flow from other populations inhabiting different environments (among-island environmental heterogeneity), as well as with local environmental heterogeneity within each population. Furthermore, local adaptation, manifested in the correlation between development time and the degree of pool drying on the islands, appears to have been caused by divergent selection pressures. The local adaptation in development time and phenotypic plasticity is quite remarkable, because the populations are young (less than 300 generations) and substantial gene flow is present among islands.

KEY WORDS: **Gene flow, genetic drift, life-history evolution, local adaptation, natural selection, phenotypic plasticity.**

Evolutionary theory predicts that adaptive divergence between populations reflects a balance between the diversifying effects of selection and the homogenizing effects of gene flow (e.g., Mayr 1963; Slatkin 1987). High gene flow constrains adaptive divergence of populations by reducing the independence of their gene pools (Räsänen and Hendry 2008) and numerous empirical studies have shown that gene flow constrains adaptive divergence in locally adapted populations (Riechert 1993; Langerhans et al. 2003; Hendry and Taylor 2004; Nosil and Crespi 2004; Crispo et al. 2006). However, high gene flow might also result in selection for phenotypic plasticity (a genotype expressing different phenotypes in different environments), because gene flow increases the likelihood that a migrating individual will disperse to an environment it is not adapted to (DeWitt and Scheiner 2004). In a landscape, where connected populations inhabit different environments, theoretical models have shown that a flexible, rather than fixed, phenotype is selected for (Sultan and Spencer 2002). Therefore, understanding the role of gene flow has particular relevance for our understanding of local adaptation and phenotypic plasticity in natural populations. Although numerous studies have investigated the role of gene flow in local adaptation of specialists (e.g., Langerhans et al. 2003; Hendry and Taylor 2004), there

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have been very few empirical investigations of the importance of gene flow for the evolution of phenotypic plasticity.

The relationship between plasticity and gene flow (or dispersal) has been the topic of several theoretical studies (Via and Lande 1985; Van Tienderen 1991; Scheiner 1998; Sultan and Spencer 2002) and the general conclusion is that high dispersal rates increase the conditions under which plasticity, rather than local specialization, is likely to evolve. This is because the increased migration between populations results in an increased frequency of specialists in environments they are not adapted to, thus giving them a lower fitness than individuals with plastic genotypes (Sultan and Spencer 2002). Nevertheless, under some circumstances the evolution of phenotypic plasticity may be negatively affected by gene flow. If the degree of plasticity in populations directly corresponds with high levels of environmental heterogeneity (i.e., higher plasticity is an adaptation to a heterogeneous environment), gene flow into these populations may disrupt this local adaptation in phenotypic plasticity, simply by the immigration of maladapted specialists (Alpert and Simms 2002; Crispo 2008). Hence, phenotypic plasticity may be considered as an adaptive trait in its own right (Scheiner 1993; Pigliucci and Schmitt 1999; Leimar et al. 2006), for which gene flow is seen as the main factor that opposes local adaptation in a heterogeneous environment (Slatkin 1987; Hendry et al. 2001; Lenormand 2002; Räsänen and Hendry 2008).

Empirical data lend some support to the prediction that high dispersal rates can increase the likelihood of plasticity to evolve. In a recent meta-analysis, it was found that the degree of phenotypic plasticity in marine invertebrates was greater in species with higher dispersal rates (Hollander 2008). It has also been shown that populations of invasive species tend to be more plastic at the invasion front than in the native strongholds (Sexton et al. 2002; Niinemets et al. 2003; Yeh and Price 2004; reviewed by Richards et al. 2006), suggesting that phenotypic plasticity may facilitate the successful colonization of novel environments (Price et al. 2003; West-Eberhard 2003). The potentially negative effect of gene flow on phenotypic plasticity has received much less attention. A small number of studies have found that populations or species inhabiting environments with high local heterogeneity are more plastic than populations in more stable environments (Richter-Boix et al. 2006; Lind and Johansson 2007), suggesting that plasticity in these populations is locally adaptive, and consequently that high gene flow may impede adaptation (Alpert and Simms 2002; Crispo 2008). However, none of these studies have estimated the degree of gene flow affecting the populations.

A system with distinct populations, that are more or less connected by gene flow and which inhabit environments with different selection pressures and environmental variation would be optimal for testing the effects of gene flow on the evolution of local adaptation in traits that are plastic or genetically fixed. Such a system is readily available for study on the Baltic Sea coast of northern Sweden, where more or less geographically isolated island populations of the common frog *Rana temporaria* L. breed in freshwater rock pools that differ in drying rate (Johansson et al. 2005). The environmental conditions dictate that these frogs need to develop from tadpole to the stage of metamorphosis, before the pools dry up. A large size at metamorphosis has substantial fitness benefits later in life (Berven 1990; Altwegg and Reyer 2003), but there is a trade-off against short development time as a response to the risk of pool desiccation (Laurila and Kujasalo 1999). Because of land uplift, the populations inhabiting the islands are relatively young (23–267 generations; Johansson et al. 2005) so they are likely to have a relatively similar genetic background. Moreover, the local selection pressures do not seem to be correlated with island age, size, number of pools, the distance to the closest population, or the distance to the mainland (Lind and Johansson 2007). The populations show local adaptation to the degree of pool drying, so tadpoles from islands with temporary pools have a shorter development time than tadpoles from islands with permanent pools (Johansson et al. 2005; Lind and Johansson 2007; Lind et al. 2008). Moreover, the populations also differ in their degree of plasticity in development time. This difference seems to be related to the degree of spatial heterogeneity in pool drying regimes on the islands (Lind and Johansson 2007).

We investigated the role of gene flow in both local adaptation in development time to metamorphosis and plasticity in development time among populations inhabiting differing environments, by addressing two main questions. First, is there a positive relationship between gene flow from populations that inhabit dissimilar environments and phenotypic plasticity that follow the predictions of theoretical models (Via and Lande 1985; Van Tienderen 1991; Scheiner 1998; Sultan and Spencer 2002), or are populations adapted to the local degree of environmental heterogeneity so that the local degree of phenotypic plasticity is negatively affected by gene flow? Second, are population differences in development time and phenotypic plasticity the result of natural selection? We addressed these questions by analyzing associations between traits and migration from different environments, as well as local selective factors, and by comparing population differentiation in quantitative traits involved in local adaptation with differentiation in neutrally evolving traits.

Methodology

GENETIC ANALYSES

During 2005 and 2006, we collected up to 12 egg clumps, each corresponding to the offspring from a single female *R. temporaria*, from each of 15 islands in the archipelago of Umeå (Fig. 1, Table 1). Egg clumps were brought back to the laboratory, hatched. and the tadpoles were allowed to develop to Gosner stage 25

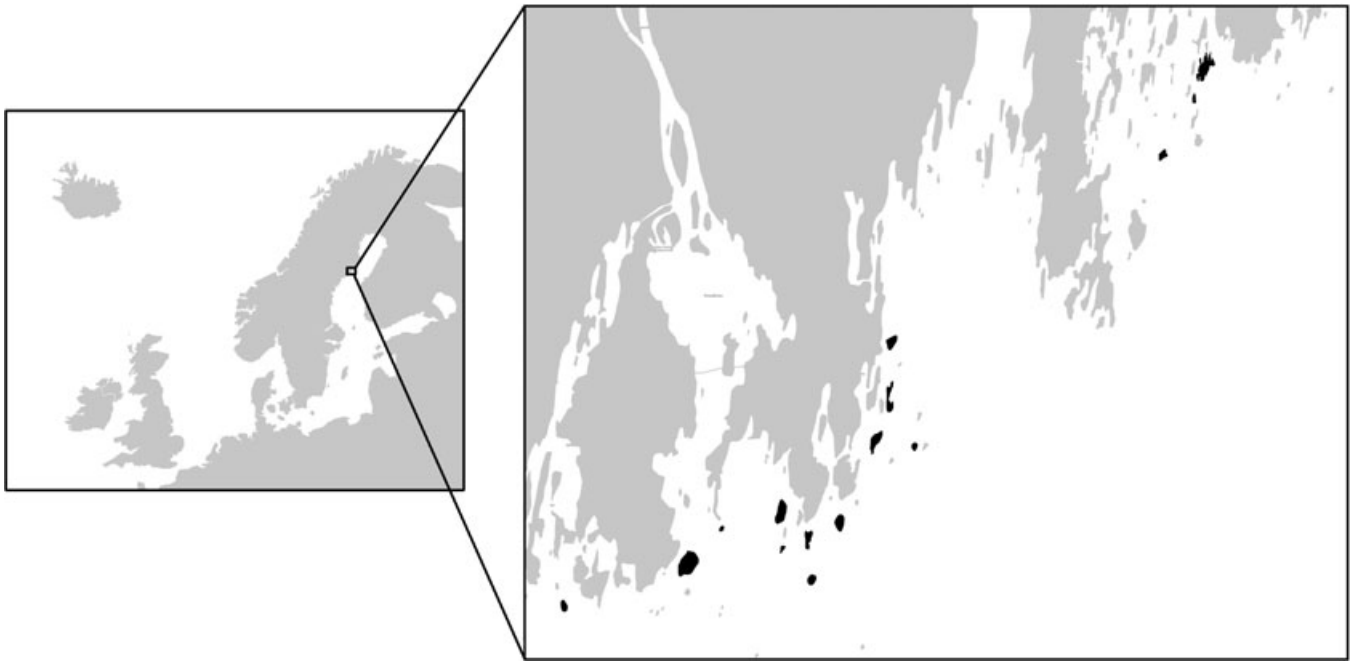

Figure 1. Locations of the 15 island populations from which molecular data were obtained.

(Gosner 1960). At this stage, one individual from each egg clump was preserved in ethanol for DNA extraction. To make genetic comparisons with mainland populations, eggs from four populations on the mainland were also collected in 2005 (Table 1), situated 66–70 km NW of the coast. Mainland populations were sampled at approximately equal distances from all island populations and far inland, to prevent any bias toward a single part of the archipelago. In total, 170 individuals were genotyped.

Template DNA was extracted using proteinase K digestion of muscle tissue, followed by salt extraction (see Aljanabi and Martinez 1997). To obtain estimates of population differentiation in neutral genetic markers, allelic variation was assessed using six

Table 1. Geographic coordinates, inbreeding coefficient (F_{IS}), allelic richness (A_R), and genetic diversity (H_S) of all sampled populations. Populations used for analyses of quantitative traits are indicated by a †.

Population	Type	Coordinates	F_{IS}	H_S	A_R
Åhällan †	island	63°40′N, 20°23′E	−0.18	0.55	2.11
Ålgrundet †	island	63°41′N, 20°25′E	0.048	0.58	2.24
Bredskär †	island	63°39′N, 20°18′E	−0.128	0.48	2.00
Buten †	island	63°43′N, 20°26′E	0.021	0.45	1.92
Fjärdgrund	island	63°40′N, 20°20′E	−0.017	0.49	2.03
Gåshällan †	island	63°39′N, 20°15′E	−0.05	0.57	2.18
Grisslögern †	island	63°47′N, 20°37′E	−0.055	0.61	2.23
Lillhaddingen	island	63°40′N, 20°24′E	−0.193	0.47	2.04
Lillklyvan †	island	63°42′N, 20°20′E	0.241	0.55	2.17
Öster Hällskär	island	63°48′N, 20°37′E	0.253	0.57	2.21
Petlandsskär	island	63°39′N, 20°24′E	0.013	0.41	1.86
Sävar-Tärnögern †	island	63°45′N, 20°36′E	0.251	0.44	1.92
Storhaddingen	island	63°40′N, 20°25′E	−0.037	0.48	2.00
Svart Lass †	island	63°41′N, 20°27′E	−0.071	0.64	2.35
Vitskär †	island	63°40′N, 20°22′E	−0.114	0.47	1.96
Nedre Mesele 1	mainland	64°15′N, 19°39′E	−0.226	0.46	1.93
Nedre Mesele 2	mainland	64°14′N, 19°39′E	0.015	0.51	2.11
Välvkälen	mainland	64°13′N, 19°38′E	0.123	0.41	1.81
Vindeln	mainland	64°13′N, 19°42′E	0.064	0.52	2.09

microsatellite loci: Rt2Ca2–22 (Lesbarrères et al. 2005), RRD590 (Vos et al. 2001), RtμH (Pidancier et al. 2002), RtU4 (Berlin et al. 2000), Rtempμ4, and Rtempμ7 (Rowe and Beebee 2001). Polymerase chain reaction (PCR) was performed using a locus-specific forward primer, tailed with an M13 universal tail (Schuelke 2000). For most loci, we used a cycling protocol of 94°C for 3 min, followed by 35 cycles of 95°C, 55°C, and 72°C, each step lasting 30 sec. For the primer pair RtμH, a lower annealing temperature of 50°C was used. The M13-tailed PCR products were used as template in a second PCR run, but replacing the forward-M13 primer with a fluorescent M13-tagged dye. The PCR conditions were otherwise as described above and the cycle was repeated eight times. The amplified microsatellite fragments were analyzed using a Beckman-Coulter CEQ8000 capillary sequencer (Beckman-Coulter, Fullerton, CA), with the GenomeLab^{TM} DNA Size Standard Kit—400 as internal size standard. The resulting chromatograms were visualized and analyzed using CEQ 8000 Fragment Analysis software (Beckman Coulter Inc., Fullerton, CA).

ANALYSES OF QUANTITATIVE TRAITS

Analyses of population differentiation in quantitative traits (Q_{ST}) are based upon the relationship between within-population and between-population variances (Spitze 1993). These are very sensitive to factors that increase variance between populations (Pujol et al. 2008). Therefore, we used individuals from 10 of the 14 island populations described in Lind and Johansson (2007), raised in the same thermo-constant room with identical environmental conditions. The remaining four populations were raised in a different thermo-constant room.

The sampling procedure and common garden experimental design used are described in detail elsewhere (Lind and Johansson 2007). Briefly, up to 10 egg clumps (each egg clump corresponding to the offspring of one female) from each of 10 islands were collected on the 2nd and 6th of May, 2005, and brought to the laboratory. To control for egg-size mediated maternal effects (Laugen et al. 2002), 10 eggs from each female were photographed and egg sizes were measured from the digital image. When hatched, at Gosner stage 25 (Gosner 1960), the tadpoles were placed in individual plastic containers (9.5 cm × 9.5 cm, height 10 cm), filled with modified tap water (aged and aerated in the presence of leaf litter). The water was replaced every fourth day, before feeding. In the common garden experiment, the temperature was set to 22°C and the tadpoles were fed ad libitum every fourth day on a mixture (1:2) of finely ground fish flakes and rabbit chow. To estimate the degree of phenotypic plasticity in frog development time as a response to the pool drying, tadpoles were subjected to one of two treatments: either a constant water volume or simulated pool drying (designated treatments C and D, respectively). In the pool drying treatment, the initial water volume of 750 mL was lowered by 33% every fourth day. Two siblings from each female clutch were individually raised under each water level treatment. The experiment was terminated at Gosner stage 42 (front legs visible), and the time to reach this stage was recorded as development time. Tadpoles were also weighed to obtain an estimate of their wet weight at metamorphosis. Plasticity in development time was calculated as the mean development time for the offspring of a female under constant water level, minus the development time under the artificial pool drying treatment. There exist a number of methods to estimate relative plasticity (reviewed by Valladares et al. 2006). Our plasticity estimate is highly correlated with other commonly used estimates, hence the choice of estimate does not influence our results (correlation of the absolute plasticity estimate with the coefficient of variation of plasticity: Pearson's $r = 0.974$, and with the relative plasticity: Pearson's $r = 0.998$).

STATISTICAL ANALYSES

Pool drying

Environmental data were obtained from Lind and Johansson (2007) and were used to test if the mean and variation in pool drying influenced the mean and plasticity of development time. Pool drying was measured as the relative decrease in water level between May 2nd or 6th and June 26th 2005. Spatial heterogeneity in pool drying regimes was estimated as the coefficient of variation of drying for each pool in which frog breeding had occurred. We used the mean pool drying for each island as the response variable in all analyses in which we investigated the effect of pool drying.

Population differentiation in genetic markers

The microsatellite data were tested for deviations from Hardy–Weinberg Equilibrium (HWE) in GENEPOP 3.4, using chi-squared tests of expected and observed heterozygosity for each locus. We also tested for deviations from HWE and linkage disequilibrium in each population for each locus, using ARLEQUIN 3.11 (Excoffier et al. 2005). Mean allelic richness (A_R) and gene diversity (H_S) were calculated for each population using FSTAT 2.9.3.2. Mean allelic richness was standardized to a minimum sample size of four individuals, using rarefaction methods (Petit et al. 1998) because A_R is especially sensitive to differences in sample size (Leberg 2002). Because A_R is highly dependent on effective population size, while H_S is much less so, a lack of correlation between the two measures can be used to identify historical processes such as bottlenecks (Widmer and Lexer 2001). We investigated if A_R and H_S were correlated, using Pearson's product-moment correlation.

Population differentiation in neutral markers (global F_{ST} and pairwise F_{ST} values between all population pairs) was analyzed in FSTAT 2.9.3.2 (Goudet 1995), using the method of Weir and Cockerham (1984). This method of estimating pairwise F_{ST} is

appropriate when they are to be compared with pairwise Q_{ST} values, because variance among groups is calculated in the same way (Whitlock 2008). The significance level of global F_{ST} was determined using a G-test, with 1600 randomizations. The 95% confidence interval of F_{ST} was estimated by bootstrapping over loci.

Population substructure between the 15 islands was investigated with the Bayesian clustering software STRUCTURE 2.3.1. (Pritchard et al. 2000), which assigns each genotyped individual to one of K populations based on allele frequencies. The number of possible populations (K) was set from 1 to 16, where each K was run with 400,000 iterations after a burn in period of 100,000 iterations. The default admixture model was used in the analysis. The optimal number of clusters was determined from the posterior probabilities generated by STRUCTURE and confirmed using the method of Evanno et al. (2005), which applies an ad hoc statistic, ΔK, based on the rate of change between successive K-values.

If dispersal is spatially limited, genetic differentiation is expected to increase with increasing distance between populations (Wright 1943). Therefore, evidence for isolation by distance was investigated by regressing pairwise values of $F_{ST}/(1 - F_{ST})$ against the logarithms of the Euclidean distances between the populations (calculated from geographical coordinates). This analysis was performed using both the full dataset and the subset of data solely for the island populations.

Population differentiation might not be a result of migration limitation; it may also arise from long-term population isolation in the absence of gene flow. In the latter case, genetic differentiation is expected to increase with increasing time in isolation. The archipelago in this study has been subject to land uplift since the last glaciation, so the age of each island is reflected in its highest elevation above sea level. We therefore calculated all pairwise isolation times between all island pairs, as the sum of their height above current sea level. We then investigated if this was correlated with the pairwise differences in F_{ST} values. The Spearman rank correlations were tested for significance using Mantel tests (1000 permutations), as implemented in the VEGAN library of the statistical package *R* (http://www.r-project.org).

Migration between the island populations may not be random and restrictions in gene flow may be related to particular barriers. To investigate the effect on genetic isolation by the distance of seawater separating populations from the mainland (hereafter referred to as seawater distance), we performed a regression analysis. The shortest seawater distance for each population was found from nautical charts (Garmin BlueChart Atlantic version 7, region EU047). Intervening islands were disregarded, assuming that they would pose an insignificant dispersal barrier compared with seawater. Pairwise F_{ST} values between all island populations and the four pooled mainland populations (66–70 km NW of the study islands) were calculated using FSTAT 2.9.3.2. To ensure that any relationship found was not simply a function of the geographical distance from the mainland populations, we also investigated if the pairwise F_{ST} values between the island populations and the mainland populations were correlated with their geographical distance, using regression methods.

Population differentiation in quantitative traits

It has been shown that, under a strictly neutral model, the between- and within-population component of variation in quantitative traits (Q_{ST}) is equal to the population differentiation in neutral loci (F_{ST}) (Spitze 1993). Q_{ST} is defined as:

$$Q_{ST} = \frac{\sigma_B^2}{2\sigma_W^2 + \sigma_B^2}, \quad (1)$$

where σ_B^2 is the between-population component of variation and σ_W^2 is the variance component within populations. A common approach to investigate adaptive divergence among populations is to compare genetic divergence for quantitative traits (Q_{ST}) with that of neutral markers (F_{ST}) (McKay and Latta 2002; Leinonen et al. 2008). The population differentiation in neutral markers reflects a balance between gene flow and drift, so factors other than genetic drift must be invoked to explain significant differences between F_{ST} and Q_{ST}. Natural selection is the most common explanation. If $Q_{ST} > F_{ST}$ it indicates that divergent or disruptive selection, favoring different trait values in different populations, is present. On the other hand, if $Q_{ST} < F_{ST}$, the populations are less differentiated than would be expected from genetic drift alone, stabilizing selection favoring the same trait value in all populations is the most likely explanation. Finally, if $Q_{ST} = F_{ST}$, the relative contributions of drift and selection to population differentiation cannot be determined (Spitze 1993; Whitlock 1999; Leinonen et al. 2008).

However, the practice of comparing F_{ST} and Q_{ST} has been criticized recently on both conceptual (Hendry 2002) and experimental grounds (Pujol et al. 2008; Whitlock 2008; Whitlock and Guillaume 2009). Conceptually, the expectation that $Q_{ST} = F_{ST}$ for traits that are not under selection hinges on the assumption that migration rates are much higher than mutation rates. For populations with low migration rates and markers with high mutation rates (e.g., microsatellites), Hendry (2002) has shown that this assumption may not be true. Moreover, when F_{ST} is high, it is more difficult to find evidence of divergent selection, as Q_{ST} is bounded between 0 and 1 (Cano et al. 2008). Experimentally, it is important to raise individuals in common garden conditions, to estimate the genetic component of the phenotypic differences (Pujol et al. 2008). Maternal effects also need to be controlled (Whitlock 2008). In addition, because traits might change in different environments, and local adaptive plasticity may be present, Q_{ST} estimations should be carried out in more than one environment, using an adequate number of populations (Whitlock 2008).

Recently Whitlock and Guillaume (2009) further noted that the comparisons between F_{ST} and Q_{ST} are problematic because in general too few loci are used to estimate the true distribution of F_{ST} and that Q_{ST} most often are not measured with high enough precision. These problems arise because estimates of Q_{ST} are compared to estimates of F_{ST}, whereas the proper null hypothesis is to compare Q_{ST} with the distribution of Q_{ST} expected for neutrally evolving traits. Whitlock and Guillaume (2009) addressed this problem by predicting the sample variance that would be expected from Q_{ST} of a neutral trait by simulating it with information on F_{ST} and the within-population additive variance of the trait.

To overcome these problems, we estimated Q_{ST} using data from a common garden experiment performed in two common environments, using individuals from 10 populations and following the $Q_{ST} - F_{ST}$ approach outlined by Whitlock and Guillaume (2009), taking maternal effects into account. Nevertheless, a number of other factors (dominance, using a full-sibling design) can also influence the estimation of within-population variation in quantitative traits and may bias our estimates of Q_{ST} downwards (Whitlock 2008).

For two quantitative traits, development time and metamorphic weight, we used the data from the common garden experiment to quantify within- and between-population variances, using the following linear model:

$$z_{ijkl} = \mu + A_i + \alpha_j + \beta_{jk} + C_{ijk} + \varepsilon_{ijkl}, \quad (2)$$

where z_{ijkl} is the phenotype of the lth individual of the kth family from the jth population. In the equation, μ is the grand mean, A_i denotes the two water level treatments, C_{ijk} is the effect of the covariate egg size, and ε_{ijkl} is the residual error term. The population (α_j) and family (β_{jk}) effects provide estimates of σ_B^2 and σ_w^2, respectively.

Because plasticity was defined as the difference in development time between the C and D treatments, plasticity could not be measured for a single individual. Hence, we estimated the plasticity in development time in all four possible pair-wise combinations of full-siblings, and then randomly picked two plasticity measures from each family, ensuring that no individual had been used to estimate both plasticity measures. This procedure was then repeated 100 times to avoid introducing bias by selecting two of the four pairs. The procedure inherently reduces the sample size to half of that used for Q_{ST} estimation of development time and metamorphic weight. Moreover, as no individual can be used twice for a plasticity estimate, we had to exclude all families in which there had been mortality (causing a reduction from 81 to 49 families, see Table S1). The consequently low statistical power of the plasticity model has to be borne in mind when interpreting the results.

We then quantified within- and between-population variances for plasticity, using the following model:

$$z_{jkl} = \mu + \alpha_j + \beta_{jk} + C_{ijk} + \varepsilon_{jkl}, \quad (3)$$

where z_{jkl} denotes the plasticity in development time. The equation differs from equation (2) only in the removal of the treatment effect.

The population (α_j) and family (β_{jk}) variance components provide estimates of σ_B^2 and σ_W^2, respectively, and were used to estimate Q_{ST} following equation (1).

To estimate the within- and between-population variance components, the models were fitted by restricted maximum-likelihood (REML) using the *lmer* function in the *lme4* library of the statistical package *R*. Because the precision of the Q_{ST} estimate may differ among methods (O'Hara and Merilä 2005), point estimates of Q_{ST} were calculated in two ways. First, Q_{ST} was calculated in the traditional way, by using the within and between population variance components obtained in equations (2) and (3) using REML. Second, we calculated Q_{ST} using a Bayesian approach, following Hall et al. (2007). In this latter method, we used the *mcmcsamp* function to sample from the posterior density of the parameters of the fitted models. Two MCMC chains of 50,000 steps were run and we assessed convergence of the chains using the library *coda*. Point estimates of Q_{ST} were then calculated as the mode of its posterior distribution.

We investigated the null hypothesis that there is no difference between the estimated Q_{ST} for each trait from the common garden experiments and the Q_{ST} of a neutrally evolving trait following the approach of Whitlock and Guillaume (2009). We started by calculating the expected among-population variance component $\hat{\sigma}_B^2$ for a purely neutral trait, which is given by the observed values of F_{ST} and within-population variance component σ_w^2 according to

$$\hat{\sigma}_B^2 = \frac{2F_{ST}\sigma_W^2}{1 - F_{ST}}. \quad (4)$$

The sampling distribution of $\hat{\sigma}_B^2$ was estimated by multiplying $\hat{\sigma}_B^2$ with a random number, drawn from a χ^2 distribution with nine degrees of freedom (number of populations minus one), which is suitable for simulating the distribution of Q_{ST} under a variety of demographic scenarios (Whitlock 2008). We then calculated the expected Q_{ST} of a neutral trait using the measured within-population variation σ_w^2 and expected among-population variation $\hat{\sigma}_B^2$, following equation (1). For each trait and population, we simulated the distribution of the test statistic, $Q_{ST} - F_{ST}$, 10,000 times, which is the null hypothesis of evolution in a purely neutral trait. We then tested if the observed $Q_{ST} - F_{ST}$ of each trait differed by the neutral expectations, by observing the quantile of the simulated distribution that had more extreme values than the

observed value, which gave us the *P*-value of the test. This was performed both for the $Q_{ST} - F_{ST}$ from the variance components estimated by REML as well as for the mode of $Q_{ST} - F_{ST}$ estimated from the Bayesian Monte-Carlo simulation (see above). R-code for the simulations is available as Supporting information.

In contrast to other methods this recently developed method has higher power to detect selection in cases with low population differentiation in neutral markers, strong selection, and a large number of populations. Using this approach, and given that F_{ST} should equal Q_{ST} for a neutral trait, $Q_{ST} - F_{ST}$ can be used as a test statistic, where the observed $Q_{ST} - F_{ST}$ difference is compared to the 95% distribution of the simulated $Q_{ST} - F_{ST}$ values.

The role of gene flow for adaptive divergence

Historic migration rates between populations were estimated using the software *MIGRATE-N 3.0* (Beerli 2008), assuming a migration matrix model with different subpopulation sizes and asymmetric migration rates between populations using a coalescent-based approach. For our mutation model, we used a continuous Brownian motion approximation to the stepwise ladder model and parameters were estimated using maximum likelihood (Beerli and Felsenstein 1999, 2001). Migration was estimated as the fraction of new immigrants in the target population from all other populations in each generation, scaled by the mutation rate. Because it is migration from populations from different environments that influence the evolution of local specialization and phenotypic plasticity (Sultan and Spencer 2002), we calculated the "migration-scaled among-island environmental heterogeneity" in pool drying regimes for each population. First, we calculated the absolute difference in mean drying regime between the target population and every other population and scaled this difference by the immigration parameter *M* between them. The among-island environmental heterogeneity for each population was then calculated as the variance in migration-scaled differences in drying regime, scaled by the mean difference in migration-scaled drying regimes (i.e., the coefficient of variation).

The effects of among-island environmental heterogeneity on development time, metamorphic weight, and plasticity in development time were then investigated using linear models of the following form:

$$z_{ij} = \mu + A_i + B_{ij} + C_{ij} + D_{ij} + \varepsilon_{ij}, \quad (5)$$

where z_{ij} is the mean phenotype of individuals from the *j*th population, μ is the grand mean, A_i denotes the two water level treatments, B_{ij} is the pool drying measure, C_{ij} is the effect of the covariate egg size, D_{ij} is the migration-scaled among-island environmental heterogeneity for the *j*th population, and ε_{ij} is the residual error term. The effect on plasticity was estimated using a model without the treatment term and with within-island environmental heterogeneity, instead of the pool drying measure. The migration-scaled among-island environmental heterogeneity was log transformed, to meet the assumption of normality, however no patterns were changed by this log transformation.

Results

HARDY–WEINBERG EQUILIBRIUM

Five of the six loci showed no significant deviation from Hardy–Weinberg Equilibrium at a global scale (Rt2Ca2–22: $\chi^2_{34} = 16.98$, $P = 0.99$; RRD590: $\chi^2_{44} = 46.44$, $P = 0.37$; RtμH: $\chi^2_{48} = 46.47$, $P = 0.54$; Rtempμ4: $\chi^2_{46} = 21.06$, $P = 1.0$; Rtempμ7: $\chi^2_{46} = 27.46$, $P = 0.99$). However, there were substantial deficiencies of heterozygotes at the locus RtU4, both globally ($\chi^2_{44} =$ infinity, $P < 0.001$) and in nine of the populations. It was therefore excluded from all further analyses. We found no pattern of linkage disequilibrium among the loci and populations. Population genetic data are presented in Table 1. Allelic richness and gene diversity (Table 1) were highly correlated ($t_{13} = 19.4$, $P < 0.001$, $r = 0.98$).

POPULATION DIFFERENTIATION

We found significant population differentiation (G-test, 1600 randomizations, $P < 0.001$) between the 15 islands. Global F_{ST} over all five loci was estimated to be 0.051 ± 0.018. Pairwise F_{ST} values ranged between 0 and 0.1828.

No population substructure was found using STRUCTURE. The posterior probabilities generated by STRUCTURE gave the best likelihood value for one population in the sample and this was not contradicted by ΔK.

There was a nonsignificant trend toward isolation by distance between all populations (islands and mainland, Mantel test, $r = 0.1833$, $P = 0.08$), and among the island populations there was no evidence of isolation by geographic distance (Mantel test, $r = -0.1977$, $P = 0.92$). However, we found that islands were more genetically distinct from mainland populations with increasing seawater distance from the mainland (linear regression, $t_{13} = 2.38$, $P = 0.044$, $r^2 = 0.28$, Fig. 2). This was not simply a function of the geographical distance from the mainland populations ($t_{13} = 1.54$, $P = 0.15$, $r^2 = 0.15$). We found no support for a model in which islands were colonized by frogs when they emerged, with subsequent population isolation, because there was no correlation between the degree of isolation in genetic markers and the time of independent isolation (Mantel test, $r = -0.1368$, $P = 0.77$).

ADAPTIVE DIFFERENTIATION IN QUANTITATIVE TRAITS

Both development time and metamorphic weight showed evidence of adaptive divergence among the island populations, with

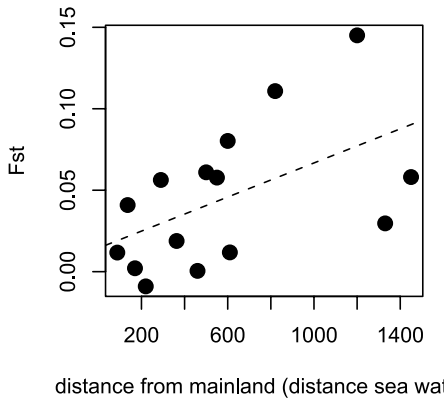

Figure 2. Regression of pairwise F_{ST} values between island populations and four pooled inland populations as a function of the shortest distance across seawater (in metres) isolating the island populations from the mainland.

$Q_{ST} - F_{ST}$ values significantly larger than $Q_{ST} - F_{ST}$ values for a neutrally evolving trait (Figs. 3A, C). A similar pattern was also found for plasticity in development time. However, the test statistics Q_{ST-} F_{ST}, was not significantly different from the values simulated for a neutral trait (Fig. 3B), hence we could not confidently differentiate between the relative contributions of genetic drift and divergent selection to the population differentiation in this trait. Estimating Q_{ST} using a Bayesian approach gave qualitatively the same result; although $Q_{ST} - F_{ST}$ for plasticity in this case was significant (Fig. S1). However, the point estimate of Q_{ST} for plasticity was less reliable than the same estimate using REML, due to a very flat posterior distribution of Q_{ST}s.

THE ROLE OF AMONG- AND WITHIN-ISLAND ENVIRONMENTAL HETEROGENEITY

The degree of plasticity in development time was positively related to the degree of environmental heterogeneity present on each island (Fig. 4A). A strong trend also suggested that the degree of phenotypic plasticity was positively influenced by the migration-scaled among-island environmental heterogeneity (Fig. 4B). The full model showed the following: within-island environmental heterogeneity, $t_6 = 4.12$, $P = 0.006$; among-island environmental heterogeneity, $t_6 = 2.26$, $P = 0.065$; egg size, $t_6 = -4.61$, $P = 0.16$; $r^2 = 0.69$.

Among-island environmental heterogeneity had no significant effect on either development time or weight at metamorphosis. The mean development time in a population was significantly affected by water-level treatment, mean pool drying on the islands and maternal effects mediated through egg size, whereas metamorphic weight was mainly influenced by the water-level treatment (Table 2).

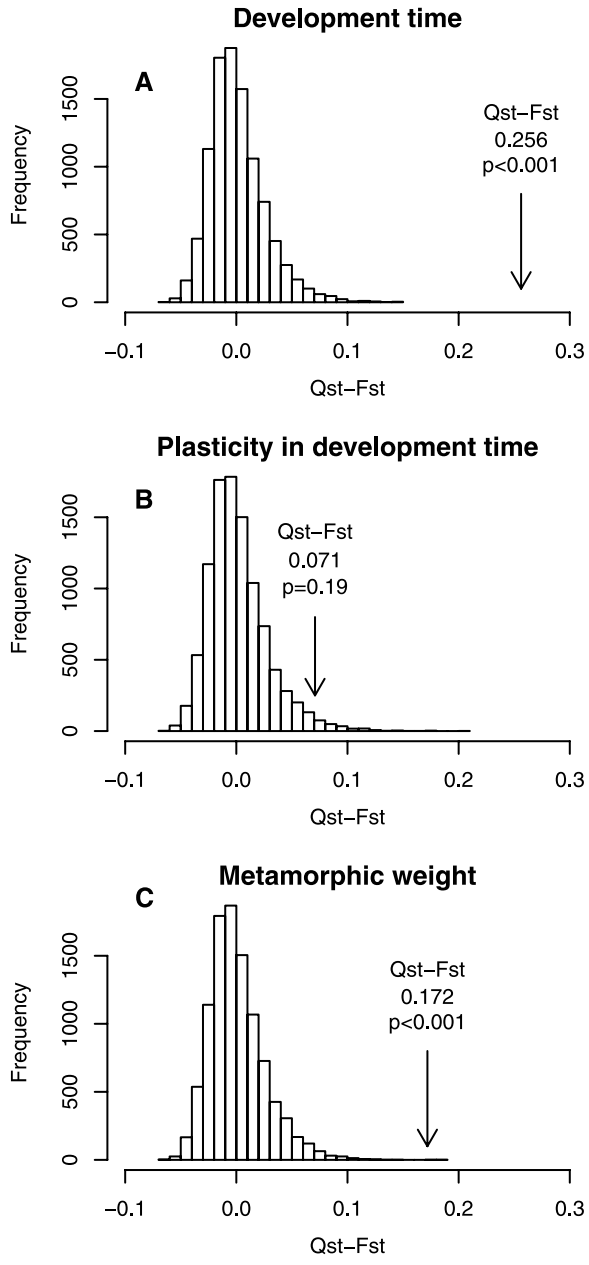

Figure 3. The simulated distribution of $Q_{ST} - F_{ST}$ for a neutral trait, and the observed point estimates of $Q_{ST} - F_{ST}$ in development time (A), plasticity in development time (B), and metamorphic weight (C) of the 10 island populations for which phenotypic data were obtained. The distribution of $Q_{ST} - F_{ST}$ for a neutrally evolving trait was simulated following Whitlock and Guillaume (2009) based upon the observed population differentiation in neutral markers (F_{ST}) and the within-population variance in each trait. The arrow indicates the observed $Q_{ST} - F_{ST}$ in each trait, and the *P*-value was obtained by observing the quantile of the simulated $Q_{ST} - F_{ST}$ distribution that had more extreme values than the observed value of $Q_{ST} - F_{ST}$.

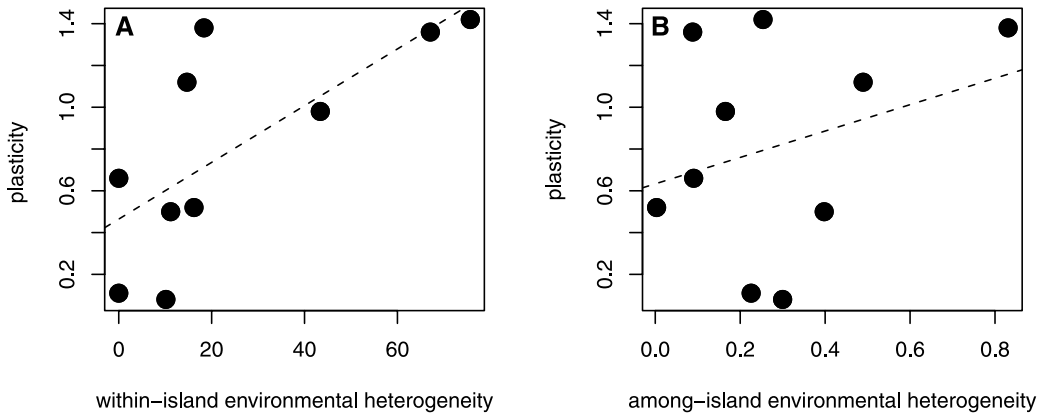

Figure 4. **The mean phenotypic plasticity in development time present in a population as a function of (A) the within-island environmental heterogeneity in pool drying regimes and (B) the migration-scaled among-island environmental heterogeneity, estimated as the coefficient of variation of the migration-scaled differences in pool drying regimes between each population and all other populations connected to it by migration.**

Discussion

We found that the degree of phenotypic plasticity in island populations of *R. temporaria* was positively correlated with the gene flow from other populations inhabiting different environments, as well as with the local environmental heterogeneity associated with each population. Furthermore, the local adaptation in development time to the degree of pool drying present on the islands was caused by the action of divergent selection.

PLASTICITY AND GENE FLOW

High gene flow is expected to increase plasticity in a set of populations, if they inhabit different environments (Sultan and Spencer 2002; Hollander 2008). However, if some populations live in more locally heterogeneous habitats than others, plasticity may instead evolve within those populations and gene flow could thus act against this local adaptation (Alpert and Simms 2002). In the island system we studied here, we found that the degree of plasticity was correlated with local environmental heterogeneity, suggesting that plasticity is a local adaptation driven by natural selection. Furthermore, we found support for the hypothesis that the degree of plasticity in the populations increases with higher rates of immigration from islands that have dissimilar environments (provided that the immigrants survive sufficiently long to contribute to the gene pool). Correspondingly, the degree of plasticity did not increase with higher rates of immigration from populations on islands that have similar environments, and this is most likely due to the migrant individuals already being adapted to those environmental conditions. Moreover, in the Q_{ST} analysis, we also found a pattern, although not significant, suggesting that divergent selection is acting on phenotypic plasticity. Thus, the overall picture from our analyses (the matching of phenotypic plasticity to degree of local environmental heterogeneity, the positive relationship between migration-scaled among island environmental heterogeneity and plasticity and the Q_{ST} analysis) strongly suggests divergent selection and local adaptation in phenotypic plasticity among the island populations. Given that phenotypic plasticity is likely to evolve as a response to temporal variation (Moran 1992), and that temporary pools often show greater variation than permanent pools over time (Newman 1992), one might also expect to find a positive relationship between the plasticity in development time and the pool drying regime. However, no such pattern is found in this system (Lind and Johansson 2007), instead it is the spatial environmental heterogeneity that is important for the evolution of plasticity.

Table 2. **Results of multiple regression analysis of the effects of the estimated levels of migration-scaled among-island environmental heterogeneity, pool drying treatment (constant or artificial pool drying), the degree of pool drying on the islands and maternal effects estimated through egg size on mean development time and metamorphic weight on the island populations. The analysis used 15 degrees of freedom for the error term. The explanatory power (r^2) was 0.67 for the model of development time and 0.87 for the metamorphic weight model. Significant coefficients are indicated by asterisks.**

	Development time		Metamorphic weight	
Coefficient	*t*	*P*	*t*	*P*
Intercept	7.81	<0.001*	4.34	<0.001*
Treatment	−2.42	0.03*	−11.6	<0.001*
Pool drying	2.83	0.003*	−1.61	0.13
Among-island heterogeneity	−1.64	0.12	0.43	0.67
Egg size	−4.03	0.001*	−1.47	0.16

The finding that both within- and between-population environmental heterogeneity results in selection for plasticity highlights the importance of gene flow for the evolution of phenotypic plasticity. Plasticity is selected for when there is migration among pools of different drying regimes within an island (within-island heterogeneity) and when there is migration into the population from islands with different pool drying regimes (among-island heterogeneity). *Rana temporaria* is able to disperse among islands in brackish seawater (Seppä and Laurila 1999) and individuals may move up to 950 m between ponds in continuous landscapes (Baker and Halliday 1999). The islands investigated here are small (9–38 ha) and pools with different drying regimes are often located only tens of meters from each other. Considering that the typical home range of adult *R. temporaria* is about 330 m^2 (as estimated by Loman 1994), between-pool dispersal on the islands is highly likely. It should be noted that it is the offspring of the dispersers rather than the dispersers themselves that are subject to selection for increased plasticity; developmental plasticity takes place during the larval stage, but it is the juvenile froglets (the postmetamorphic stage) that disperse. The same holds true also for labile (behavioral) plasticity in the larval stage.

Patterns of gene flow have also been invoked to explain the existence of costs of plasticity. It has been argued (Crispo 2008) that finding costs of plasticity would indicate that specialization rather than plasticity is the optimal strategy and that gene flow is working against local specialization. Considering that phenotypic plasticity is locally adaptive in this island system (Lind and Johansson 2007, this study), and that the most plastic populations are also those with the highest costs of plasticity (Lind and Johansson 2009), we do not find support for the hypothesis that local specialization is the optimal outcome. Instead, our data conform to the model of Van Tienderen (1991), according to which costs of plasticity increase with increased levels of plasticity (Lind and Johansson 2009). This model has found support in the few empirical systems where costs of plasticity have been compared among populations (Merilä et al. 2004; Lind and Johansson 2009).

LOCAL SPECIALIZATION

Matching of the development time to prevailing environmental conditions commonly occurs in amphibians both at the population level (Merilä et al. 2000; Laugen et al. 2003; Palo et al. 2003) and when species inhabiting different habitats are compared (Morey and Reznick 2000, 2004; Richter-Boix et al. 2006). We found that tadpole development is faster in more temporary island and in conjunction with observed population differentiation being higher than expected for a neutrally evolving trait (i.e., there is divergent selection), this provides strong evidence that natural selection plays a role in shaping population differentiation in frog development time.

We also found evidence for divergent natural selection acting on metamorphic weight, although with a smaller Q_{ST} than that obtained for development time. We interpret this as a correlated response to selection acting on development time. Because a large metamorphic weight has many fitness benefits (Berven 1990; Altwegg and Reyer 2003), it would most likely be maximized in all populations, were it not for the trade-off between development time and metamorphic weight (Laurila and Kujasalo 1999). In contrast to development time, we found no significant relationship between the degree of pool drying and metamorphic weight, which further supports the interpretation that selection acts mainly on development time (see also Johansson et al. 2005; Lind et al. 2008). One reason for the lack of a perfect relationship between selection on development time and correlated responses in metamorphic weight could be that metamorphic weight, which is closely connected to general fitness (Altwegg and Reyer 2003), is also subject to constraints (Morey and Reznick 2004; Lind et al. 2008).

The results of this study show that local selection pressures can lead to divergent selection, even over small geographic scales with relatively high levels of gene flow. Evidence for divergent selection is commonly found from F_{ST}/Q_{ST} analyses in systems with high gene flow (Leinonen et al. 2008 and references therein). This rapid local adaptation may appear surprising, but when diverging selection pressures act on quantitative traits among populations, linkage disequilibrium can develop among the allele frequencies at the loci underlying these traits. Therefore, trait values can change substantially, even with fairly small changes in the underlying allele frequencies. Differentiation in quantitative traits is hence decoupled from that of neutral markers (Latta 1998).

When inferring selection from analyses of F_{ST} and Q_{ST}, one should bear in mind the recent conceptual (Hendry 2002) and methodological (Pujol et al. 2008; Whitlock 2008; Whitlock and Guillaume 2009) criticisms of such comparisons. Here, our Q_{ST} estimates are based on data obtained from common garden studies and we applied more than one treatment. We also used several populations and nongenetic maternal effects were taken into account, thus avoiding the most important methodological pitfalls identified by the cited critics. Moreover, our global F_{ST} is low, although significant, which suggests there is high gene flow between populations. Because, the conceptual problems with F_{ST} estimates are mainly associated with cases of strong population differentiation, where the mutation rate might be equal to the migration rate (Hendry 2002), it is unlikely that this significantly influences the results of our study.

PATTERNS OF GENE FLOW AND COLONIZATION

With random dispersal, there is likely to be increasing genetic isolation with increasing distance between populations, because distance itself is thought to be a major factor limiting dispersal

and forms the basis of the isolation by distance model of Wright (1943). Isolation by distance patterns are often present among natural populations (e.g., Hutchison and Templeton 1999; Storz 2002), including inland *R. temporaria* populations in Sweden (Palo et al. 2003; Johansson et al. 2006). However, we did not find isolation by distance among the investigated island populations. There are three possible explanations for this absence (Keyghobadi et al. 2005; Bergek and Björklund 2009). First, gene flow may be very high, resulting in panmixia. Second, gene flow may be very low and any differentiation between populations may be a consequence of historical divergence from each other and the mainland. We find these two scenarios unlikely. The island populations are genetically differentiated, but there is evidence of both historical and current gene flow among them. The absence of a relationship between island age and population differentiation in neutral markers and the high correlation between allelic richness and gene diversity (the latter indicating no bottleneck events, Widmer and Lexer 2001), further support a model of continuous gene flow from mainland to islands.

The third explanation is that there are barriers to dispersal other than geographical distance. We identified seawater distance as a potential barrier to dispersal. The relationship was not simply a function of the total geographical distance to the inland populations and suggests that there is continuous gene flow from the mainland, explaining the absence of an isolation by distance pattern. Because other *Rana* species are able to survive in brackish water for long periods (Ruibal 1959), the low salinity of the area (0.35–0.4%) is most likely a very permeable dispersal barrier for swimming juveniles. However, as population differentiation was explained by the seawater distance, but not by the total distance (including intervening islands) from the mainland, seawater seems to be a much stronger dispersal barrier than the terrestrial habitat on the intervening islands. A similar level of population differentiation of *R. temporaria* populations to the level we found is also present in other archipelagos in the Baltic Sea (Seppä and Laurila 1999).

CONCLUSIONS

We found evidence of local adaptation in development time and phenotypic plasticity in development time in island populations of *R. temporaria*. Plasticity was selected for in populations on islands with a number of pools with different pool-drying regimes and in populations into which there was migration of individuals from populations subjected to different pool-drying regimes. Thus, this study highlights the importance of migration for the evolution of phenotypic plasticity, both at a local scale (migration between different pool types within an island) and regional scale (migration between islands with different environments).

In addition, we also found local adaptation in development time to the local pool-drying conditions. Plasticity in development time is present on the mainland (Almfeldt 2005), hence we suggest that this inherent plasticity accommodated a shift in the mean of this trait when frogs invaded islands with temporary pools, enabling them to develop sufficiently rapidly to survive in the novel environment. Subsequent adaptation may then have taken place in two steps (modeled by Lande 2009). First, a drop in mean fitness of the populations in the novel environments may have led to an increase in phenotypic plasticity, allowing the optimum phenotypes to be expressed. Second, at a slower rate the populations may also adapt to their new environments by genetic assimilation, enabling the genotypes to express appropriate phenotypes without the need for plastic induction. This essentially describes the fixation of a new phenotype through a Baldwin effect (see Baldwin 1896). Given the young age of these islands (corresponding to less than 300 *R. temporaria* generations, Johansson et al. 2005) and the small census sizes of breeding females (6–70, median = 18), the speed of this local adaptation in phenotypic plasticity and specialization is quite remarkable.

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LITERATURE CITED

Aljanabi, S. M., and I. Martinez. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucl. Acid. Res. 25:4692–4693.

Almfelt, J. E. M. 2005. Plastic acceleration of metamorphosis in the common frog (*Rana temporaria*) in response to pool drying: the effect of geographical isolation. Master Thesis. Umeå University.

Alpert, P., and E. L. Simms. 2002. The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evol. Ecol. 16:285–297.

Altwegg, R., and H. U. Reyer. 2003. Patterns of natural selection on size at metamorphosis in water frogs. Evolution 57:872–882.

Baker, J. M. R., and T. Halliday. 1999. Amphibian colonization of new ponds in an agricultural landscape. Herpet. J. 9:55–63.

Baldwin, J. M. 1896. A new factor in evolution. Am. Nat. 30:441–451.

Beerli, P. 2008. Migrate version 3.0—a maximum likelihood and Bayesian estimator of gene flow using the coalescent. Available at http://popgen.scs.edu/migrate.html (accessed April 22, 2009).

Beerli, P., and J. Felsenstein. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. Genetics 152:763–773.

———. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proc. Natl. Acad. Sci. USA 98:4563–4568.

Bergek, S., and M. Björklund. 2009. Genetic and morphological divergence reveals local subdivision of perch (*Perca fluviatilis* L.). Biol. J. Linn. Soc. 96:746–758.

Berlin, S., J. Merilä, and H. Ellegren. 2000. Isolation and characterization of polymorphic microsatellite loci in the common frog, *Rana temporaria*. Mol. Ecol. 9:1938–1939.

Berven, K. A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). Ecology 71:1599–1608.

Cano, J. M., T. Shikano, A. Kuparinen, and J. Merilä. 2008. Genetic differentiation, effective population size and gene flow in marine fishes: implications for stock management. J. Integr. Field Sci. 5:1–10.

Crispo, E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. J. Evol. Biol. 21:1460–1469.

Crispo, E., P. Bentzen, D. N. Reznick, M. T. Kinnison, and A. P. Hendry. 2006. The relative influence of natural selection and geography on gene flow in guppies. Mol. Ecol. 15:49–62.

DeWitt, T. J., and S. M. Scheiner. 2004. Phenotypic plasticity: functional and conceptual approaches. Oxford Univ. Press, Oxford, U.K.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software *STRUCTURE*: a simulation study. Mol. Ecol. 14:2611–2620.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinform. Online 1:47–50.

Gosner, K. L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183–190.

Goudet, J. 1995. *FSTAT* (Version 1.2): A computer program to calculate F-statistics. J. Hered. 86:485–486.

Hall, D., V. Luquez, V. M. Garcia, K. R. St Onge, S. Jansson, and P. K. Ingvarsson. 2007. Adaptive population differentiation in phenology across a latitudinal gradient in European aspen (*Populus tremula*, L.): a comparison of neutral markers, candidate genes and phenotypic traits. Evolution 61:2849–2860.

Hendry, A. P. 2002. $Q_{ST} > = \neq < F_{ST}$? Trends Ecol. Evol. 17:502.

Hendry, A. P., and E. B. Taylor. 2004. How much of the variation in adaptive divergence can be explained by gene flow?—an evaluation using lake-stream stickleback pairs. Evolution 58:2319–2331.

Hendry, A. P., T. Day, and E. B. Taylor. 2001. Population mixing and the adaptive divergence of quantitative traits in discrete populations: a theoretical framework for empirical tests. Evolution 55:459.

Hollander, J. 2008. Testing the grain-size model for the evolution of phenotypic plasticity. Evolution 62:1381–1389.

Hutchison, D. W., and A. R. Templeton. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898–1914.

Johansson, F., J. Hjelm, and B. E. Giles. 2005. Life history and morphology of *Rana temporaria* in response to pool permanence. Evol. Ecol. Res. 7:1025–1038.

Johansson, M., C. R. Primmer, and J. Merilä. 2006. History versus current demography: explaining the genetic population structure of the common frog (*Rana temporaria*). Mol. Ecol. 15:975–983.

Keyghobadi, N., J. Roland, and C. Strobeck. 2005. Genetic differentiation and gene flow among populations of the alpine butterfly, *Parnassius smintheus*, vary with landscape connectivity. Mol. Ecol. 14:1897–1909.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. J. Evol. Biol. 22:1435–1446.

Langerhans, R. B., C. A. Layman, A. K. Langerhans, and T. J. DeWitt. 2003. Habitat-associated morphological divergence in two Neotropical fish species. Biol. J. Linn. Soc. 80:689–698.

Latta, R. G. 1998. Differentiation of allelic frequencies at quantitative trait loci affecting locally adaptive traits. Am. Nat. 151:283–292.

Laugen, A. T., A. Laurila, and J. Merilä. 2002. Maternal and genetic contributions to geographical variation in *Rana temporaria* larval life-history traits. Biol. J. Linn. Soc. 76:61–70.

Laugen, A. T., A. Laurila, K. Räsänen, and J. Merilä. 2003. Latitudinal countergradient variation in the common frog (*Rana temporaria*) development rates—evidence for local adaptation. J. Evol. Biol. 16:996–1005.

Laurila, A., and J. Kujasalo. 1999. Habitat duration, predation risk and phenotypic plasticity in common frog (*Rana temporaria*) tadpoles. J. Anim. Ecol. 68:1123–1132.

Leberg, P. L. 2002. Estimating allelic richness: effects of sample size and bottlenecks. Mol. Ecol. 11:2445–2449.

Leimar, O., P. Hammerstein, and T. J. M. Van Dooren. 2006. A new perspective on developmental plasticity and the principles of adaptive morph determination. Am. Nat. 167:367–376.

Leinonen, T., R. B. O'Hara, J. M. Cano, and J. Merilä. 2008. Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. J. Evol. Biol. 21:1–17.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends Ecol. Evol. 17:183–189.

Lesbarrères, D., C. R. Primmer, A. Laurila, and J. Merilä. 2005. Environmental and population dependency of genetic variability-fitness correlations in *Rana temporaria*. Mol. Ecol. 14:311–323.

Lind, M. I., and F. Johansson. 2007. The degree of phenotypic plasticity is correlated with the spatial environmental heterogeneity experienced by island populations of *Rana temporaria*. J. Evol. Biol. 20:1288–1297.

———. 2009. Costs and limits of phenotypic plasticity in island populations of the common frog *Rana temporaria* under divergent selection pressures. Evolution 63:1508–1518.

Lind, M. I., F. Persbo, and F. Johansson. 2008. Pool desiccation and developmental thresholds in the common frog, *Rana temporaria*. Proc. R. Soc. Lond. B 275:1073–1080.

Loman, J. 1994. Site tenacity, within and between summers, of *Rana arvalis* and *Rana temporaria*. Alytes 12:15–29.

Mayr, E. 1963. Animal species and evolution. Harvard Univ. Press, Cambridge.

McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. Trends Ecol. Evol. 17:285–291.

Merilä, J., A. Laurila, and B. Lindgren. 2004. Variation in the degree and costs of adaptive phenotypic plasticity among *Rana temporaria* populations. J. Evol. Biol. 17:1132–1140.

Merilä, J., A. Laurila, A. T. Laugen, K. Räsänen, and M. Pahkala. 2000. Plasticity in age and size at metamorphosis in *Rana temporaria*-–comparison of high and low latitude populations. Ecography 23:457–465.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. Am. Nat. 139:971–989.

Morey, S., and D. N. Reznick. 2000. A comparative analysis of plasticity in larval development in three species of spadefoot toads. Ecology 81:1736–1749.

———. 2004. The relationship between habitat permanence and larval development in California spadefoot toads: field and laboratory comparisons of developmental plasticity. Oikos 104:172–190.

Newman, R. A. 1992. Adaptive plasticity in amphibian metamorphosis: what type of phenotypic variation is adaptive, and what are the costs of such plasticity. BioScience 42:671–678.

Nielsen, R., and J. Wakeley. 2001. Distinguishing migration from isolation: a Markov Chain Monte Carlo approach. Genetics 158:885–896.

Niinemets, Ü., F. Valladares, and R. Ceulemans. 2003. Leaf-level phenotypic variability and plasticity of invasive *Rhododendron ponticum* and non-invasive *Ilex aquifolium* co-occurring at two contrasting European sites. Plant, Cell Environ. 26:941–956.

Nosil, P., and B. Crespi. 2004. Does gene flow constrain adaptive divergence or vice versa? A test using ecomorphology and sexual isolation in *Timema cristinae* walking-sticks. Evolution 58:102.

O'Hara, R. B., and J. Merilä. 2005. Bias and precision in Q_{ST} estimates: problems and some solutions. Genetics 171:1331–1339.

Palo, J. U., R. B. O'Hara, A. T. Laugen, A. Laurila, C. R. Primmer, and J. Merilä. 2003. Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative genetic data. Mol. Ecol. 12:1963–1978.

Petit, R. J., A. E. Mousadik, and O. Pons. 1998. Identifying populations for conservation on the basis of genetic markers. Cons. Biol. 12:844–855.

Pidancier, N., P. Gauthier, C. Miquel, and F. Pompanon. 2002. Polymorphic microsatellite DNA loci identified in the common frog (*Rana temporaria*, Amphibia, Ranidae). Mol. Ecol. Notes 2:304–305.

Pigliucci, M., and J. Schmitt. 1999. Genes affecting phenotypic plasticity in Arabidopsis: pleiotropic effects and reproductive fitness of photomorphogenic mutants. J. Evol. Biol. 12:551–562.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure from multilocus genotype data. Genetics 155:945–959.

Price, T. D., A. Qvarnström, and D. E. Irwin. 2003. The role of phenotypic plasticity in driving genetic evolution. Proc. R. Soc. Lond. B 270:1433–1440.

Pujol, B., A. J. Wilson, R. I. C. Ross, and J. R. Pannell. 2008. Are Q_{ST}-F_{ST} comparisons for natural populations meaningful? Mol. Ecol. 17:4782–4785.

Räsänen, K., and A. P. Hendry. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. Ecol. Lett. 11:624–636.

Richards, C. L., O. Bossdorf, N. Z. Muth, J. Gurevitch, and M. Pigliucci. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecol. Lett. 9:981–993.

Richter-Boix, A., G. A. Llorente, and A. Montori. 2006. A comparative analysis of the adaptive developmental plasticity hypothesis in six Mediterranean anuran species along a pond permanency gradient. Evol. Ecol. Res. 8:1139–1154.

Riechert, S. E. 1993. Investigation of potential gene flow limitation of behavioral adaptation in an aridlands spider. Behav. Ecol. Sociobiol. 32:355–363.

Rowe, G., and T. J. C. Beebee. 2001. Polymerase chain reaction primers for microsatellite loci in the common frog *Rana temporaria*. Mol. Ecol. Notes 1:6–7.

Ruibal, R. 1959. The ecology of a brackish water population of *Rana pipiens*. Copeia 1959:315–322.

Scheiner, S. M. 1993. Plasticity as a selectable trait: reply to Via. Am. Nat. 142:371–373.

———. 1998. The genetics of phenotypic plasticity. VII. Evolution in a spatially-structured environment. J. Evol. Biol. 11:303–320.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. 18:233–234.

Seppä, P., and A. Laurila. 1999. Genetic structure of island populations of the anurans *Rana temporaria* and *Bufo bufo*. Heredity 82:309–317.

Sexton, J. P., J. K. McKay, and A. Sala. 2002. Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. Ecol. Appl. 12:1652–1660.

Slatkin, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

Spitze, K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. Genetics 135:367–374.

Storz, J. F. 2002. Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analysis of clinal variation. Mol. Ecol. 11:2537–2551.

Sultan, S. E., and H. G. Spencer. 2002. Metapopulation structure favours plasticity over local adaptation. Am. Nat. 160:271–283.

Valladares, F., D. Sanchez-Gomez, and M. A. Zavala. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J. Ecol. 94:1103–1116.

Van Tienderen, P. H. 1991. Evolution of generalists and specialists in spatially heterogeneous environments. Evolution 45:1317–1331.

Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505–522.

Vos, C. C., A. G. Antonisse-De Jong, P. W. Goedhart, and M. J. M. Smulders. 2001. Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). Heredity 86:598–608.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370.

West-Eberhard, M. J. 2003. Developmental plasticity and evolution. Oxford Univ. Press Inc, New York, USA.

Whitlock, M. C. 2008. Evolutionary inference from Q_{ST}. Mol. Ecol. 17:1885–1896.

———. 1999. Neutral additive genetic variance in a metapopulation. Genet. Res. 74:215–221.

Whitlock, M. C., and F. Guillaume. 2009. Testing for spatially divergent selection: comparing Q_{ST} to F_{ST}. Genetics 183:1055–1063.

Widmer, A., and C. Lexer. 2001. Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. Trends Ecol. Evol. 16:267–269.

Wright, S. 1943. Isolation by distance. Genetics 28:114–138.

Yeh, P. J., and T. D. Price. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. Am. Nat. 164:531–542.

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Supporting Information

The following supporting information is available for this article:

Figure S1. The simulated distribution of $Q_{ST} - F_{ST}$ for a neutral trait, and the observed point estimates of $Q_{ST} - F_{ST}$ in development time (a), plasticity in development time (b), and metamorphic weight (c) of the 10 island populations for which phenotypic data were obtained.

Table S1. Mean and standard error of the metamorphic weight (g), development time (days), and plasticity in development time (days) for all islands, together with the number of families used (n_f) and the number of families without any mortality, used for the analysis of Q_{ST} of plasticity in development time.

File S1. R-code used for simulating the distribution of $Q_{ST} - F_{ST}$ for a neutral trait, and estimating the quantile of the simulated distribution that has more extreme values than the observed value of $Q_{ST} - F_{ST}$.

Supporting Information may be found in the online version of this article.