Molecular Ecology (2003) doi: 10.1046/j.1365-294X.2003.01865.x

Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative genetic data

J. U. PALO,* R. B. O'HARA,*† A. T. LAUGEN,‡ A. LAURILA,‡ C. R. PRIMMER§ and J. MERILÄ*
*Ecological Genetics Research Unit, Department of Ecology and Systematics, PO Box 65, FIN-00014 University of Helsinki, Finland, †Rolf Nevanlinna Institute, PO Box 4, FIN-00014 University of Helsinki, Finland, ‡Department of Population Biology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18 d, SE-752 36 Uppsala, Sweden, §Department of Ecology and Systematics, PO Box 65, FIN-00014 University of Helsinki, Finland

Abstract

The relative roles of natural selection and direct environmental induction, as well as of natural selection and genetic drift, in creating clinal latitudinal variation in quantitative traits have seldom been assessed in vertebrates. To address these issues, we compared molecular and quantitative genetic differentiation between six common frog (*Rana temporaria*) populations along an approximately 1600 km long latitudinal gradient across Scandinavia. The degree of population differentiation ($Q_{ST} \approx 0.81$) in three heritable quantitative traits (age and size at metamorphosis, growth rate) exceeded that in eight (neutral) microsatellite loci ($F_{ST} = 0.24$). Isolation by distance was clear for both neutral markers and quantitative traits, but considerably stronger for one of the three quantitative traits than for neutral markers. Q_{ST} estimates obtained using animals subjected to different rearing conditions (temperature and food treatments) revealed some environmental dependency in patterns of population divergence in quantitative traits, but in general, these effects were weak in comparison to overall patterns. Pairwise comparisons of F_{ST} and Q_{ST} estimates across populations and treatments revealed that the degree of quantitative trait differentiation was not generally predictable from knowledge of that in molecular markers. In fact, both positive and negative correlations were observed depending on conditions where the quantitative genetic variability had been measured. All in all, the results suggest a very high degree of genetic subdivision both in neutral marker genes and genes coding quantitative traits across a relatively recently (< 9000 years) colonized environmental gradient. In particular, they give evidence for natural selection being the primary agent behind the observed latitudinal differentiation in quantitative traits.

Keywords: amphibians, Bayesian statistics, F_{ST}, geographical variation, microsatellite DNA, Q_{ST}

Received 2 January 2003; revision received 17 March 2003; accepted 18 March 2003

Introduction

Intraspecific studies of geographical variation have provided some of the most compelling evidence for the occurrence of microevolution as a response to spatially varying selection pressures (e.g. Endler 1977; Conover & Schultz 1995; Gilchrist & Partridge 1999; Huey *et al*. 2000). However, although studies of geographical variation have proved valuable, especially when combined with common garden or transplant experiments (e.g. Linhart & Grant 1996; Reznick *et al*. 1997), a general problem with them is that the actual selection regimes of different populations are seldom known. Consequently, even in cases where the genetic basis for quantitative trait differentiation among populations has been established, the role of natural selection, as opposed to stochastic forces such as genetic drift, as a driving force behind the observed differentiation has typically remained a matter of educated speculation, rather than established fact. Fortunately, comparisons of

Correspondence: Juha Merilä. Fax: + 358-9-191 57694; E-mail: juha.merila@helsinki.fi

© 2003 Blackwell Publishing Ltd

divergence in neutral marker genes (as measured by F_{ST}) and genes coding quantitative traits (as measured by Q_{ST}; Wright 1951) now allow for an assessment of the relative importance of natural selection and genetic drift as a cause of population differentiation in quantitative traits (e.g. Rogers 1986; Merilä & Crnokrak 2001; McKay & Latta 2002). Higher relative divergence in the quantitative traits than in neutral markers (i.e. $Q_{ST} > F_{ST}$) is indicative of directional selection favouring different genotypes in different populations, whereas the opposite (i.e. $Q_{ST} < F_{ST}$) suggests that the same genotypes are favoured in different populations, i.e. stabilizing selection. However, if the two measures do not differ significantly, the relative effects of drift and selection on population differentiation cannot be separated (Merilä & Crnokrak 2001).

Although comparative studies of molecular and quantitative genetic differentiation are still scarce, they suggest a major role for directional natural selection as a driving force behind intraspecific differentiation in quantitative traits (reviews in Merilä & Crnokrak 2001; Crnokrak & Merilä 2002; McKay & Latta 2002). Together with other similar approaches (Rieseberg *et al.* 2002; see also Gockel *et al.* 2001; Koskinen *et al.* 2002), they provide an example of how molecular and quantitative genetic methods can be used in combination to address fundamental questions in evolutionary biology. Comparative studies of quantitative trait and neutral marker divergence are also interesting from a conservation genetics perspective as management decisions often rest on population genetic analyses conducted with neutral molecular markers (e.g. Moritz *et al.* 1995; Haig 1998; Reed & Frankham 2001). For instance, operational definitions of evolutionary significant units (ESUs) are based on divergence in neutral or nearly neutral markers (Moritz 1994; Moritz *et al.* 1995; but see also Fraser & Bernatchez 2001). However, quantitative characters are more likely to be related to population persistence and may therefore be more relevant for conservation (Lynch 1996; Storfer 1996). Nevertheless, the question of whether the levels of variation and the degree of population differentiation in neutral marker genes is correlated with levels of variation and the degree of population differentiation in genes coding quantitative traits remains contentious (Pfender *et al.* 2000; Merilä & Crnokrak 2001; Reed & Frankham 2001; Latta & McKay 2002). In a meta-analysis of 18 studies reporting Q_{ST} and F_{ST} values on 20 species, Merilä & Crnokrak (2001) did indeed find a positive correlation between the two divergence indices across different studies (see also Crnokrak & Merilä 2002; Latta & McKay 2002; McKay & Latta 2002). However, studies comparing the predictive power of neutral markers as indicators of divergence in quantitative traits at the intraspecific level are still lacking (but see Morgan *et al.* 2001; Steinger *et al.* 2002), as are studies examining the sensitivity of Q_{ST} estimates to genotype–environment interactions.

The aim of this study was to evaluate the importance of genetic drift and natural selection in explaining genetic differentiation in three quantitative traits between six common frog, *Rana temporaria*, populations situated along a 1600 km latitudinal gradient across Scandinavia. In particular, we were interested to determine whether the divergence in quantitative traits exceeds that in neutral marker genes (i.e. if $Q_{ST} > F_{ST}$), as would be expected if the divergence in the quantitative traits (i.e. age, size and growth rate until metamorphosis) has been driven by natural selection. As an additional test of selection-driven differentiation, we compared the degree of isolation by distance in marker and quantitative traits, the expectation under selective differentiation being that the correlation between geographical distance and differentiation should be higher for quantitative traits than for molecular markers. This prediction applies naturally only for those quantitative traits which show latitudinally-ordered divergence in their trait means. Furthermore, we evaluated the hypothesis that the degree of differentiation in molecular markers is predictive of degree of differentiation in quantitative traits by pairwise comparisons of population differentiation in F_{ST} and Q_{ST} estimates. Finally, we evaluated the sensitivity of our conclusions to the environmental conditions under which the quantitative trait divergence was estimated by estimating population divergence and additive genetic variances in quantitative traits under different environmental conditions (treatments).

Materials and methods

The study species and populations

The common frog is the most widespread anuran in Europe, exhibiting extensive geographical variation in larval and adult life history traits (Fog *et al.* 1997; Miaud *et al.* 1999; Miaud & Merilä 2001). A number of common garden studies (Merilä *et al.* 2000a; Laugen *et al.* 2002; Laurila *et al.* 2002; Pahkala *et al.* 2002) have demonstrated genetic differentiation in larval traits across the Scandinavian peninsula. However, evidence for a possible clinal and adaptive nature of this differentiation remains elusive, since most of these studies (but see Pahkala *et al.* 2002; Laugen *et al.* 2003a,b) are based on comparisons of single low and high latitude populations, and direct evidence for involvement of natural selection is lacking. Nevertheless, there is a reason to expect latitudinally-ordered selection favouring faster developing and growing larvae in the north because of the rapidly shortening growth season from south to north (Laugen *et al.* 2003a).

The six populations included in this study were situated along a latitudinal gradient from southern Sweden to northern Finland (Fig. 1; Table 1). These populations are the same as those used by Laugen *et al.* (2003a), except for an additional population from northern Sweden (Ammarnäs). More information about populations and the climatic

Table 1 Study populations and summary of genetic variability measures

Population	Coordinates	N	A	H_E	F_{IS}
Lund	55°42′ N, 13°26′ E	36 (33.3)	8.6	0.686	0.088
Uppsala	59°51′ N, 17°14′ E	42 (36.5)	5.3	0.576	0.003
Umeå	63°49′ N, 20°14′ E	36 (35.9)	4.3	0.594	−0.050
Ammarnäs	65°54′ N, 16°18′ E	36 (30.4)	5.5	0.504	−0.157
Kiruna	67°51′ N, 21°02′ E	36 (35.1)	5.2	0.658	−0.011
Kilpisjärvi	69°03′ N, 20°47′ E	42 (39.9)	5.2	0.601	−0.061
All		192 (35.2)	8.7	0.618	−0.023

N: number of individuals studied (average number of genotyped individuals per locus); A: mean allelic richness per locus (based on minimum sample size of 23 individuals); H_E: expected mean heterozygosity; F_{IS}: fixation index indicating deviations from H–W expectations (in all cases $P > 0.05$).

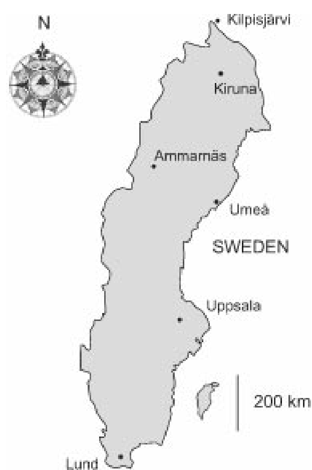

Fig. 1 Map showing the location of the six study populations.

conditions in these localities is given in Merilä *et al*. (2000b) and Laugen *et al*. (2003a,b).

Molecular genetic data and analyses

To obtain estimates of population differentiation (F_{ST}) in neutral markers, allelic variation was assessed for 36–42 randomly-sampled adult individuals from each of the six populations in eight microsatellite loci: Rt2Ca2–22, Rt2Ca25 (T. Garner, unpublished), RRD590 (Vos *et al*. 2001), RtμH (Pidancier *et al*. 2001), RtSB03 (Berlin *et al*. 2000), Rtempμ4, Rtempμ5 and Rtempμ7 (Rowe & Beebee 2001). DNA was extracted using standard sodium dodecyl sulphate (SDS)–proteinase K digestion treatment followed by isopropanol precipitation (e.g. Bruford *et al*. 1992). Polymerase chain reaction (PCR) amplifications were performed in a total volume of 10 μL using approximately 100 ng of genomic DNA, 250 μM of each dNTP, 1 × PCR buffer (PE Biosystems), 1.5 mM (4 mM in RtSB03) of $MgCl_2$, 0.25 U AmpliTaq DNA polymerase (PE Biosystems) and 100–600 nM of each primer, one of which was end-labelled with a fluorescent dye. The cycling profiles used for most of the loci consisted of 94 °C for 3 min (initial denaturation) and 35 cycles of 94 °C, 55 °C and 72 °C (30 s each), and an additional elongation step of 5 min at 72 °C. For loci RtμH and RtSB03, a lower annealing temperature of 50 °C was used. Locus Rtempμ5 was amplified using a touchdown profile, where the annealing temperature was gradually lowered from 50 °C to 40 °C during the first 20 cycles, and kept constant for the last 10 cycles. The PCR products for each individual were resolved by denaturing polyacrylamide gel electrophoresis (PAGE) (5% LongRanger gel, BMA, Rockland, USA) on a single lane using an ABI 377 DNA sequencer. Data collection and allele scoring were performed using the GENESCAN 3.1 and GENOTYPER 2.5 software.

Allele frequency, allelic richness (El Mousadik & Petit 1996), observed heterozygosity and unbiased estimates of expected heterozygosity (Nei 1987) were calculated for each population. Deviations from Hardy–Weinberg equilibrium within populations at each locus were assessed by calculating F_{IS}, the statistical significance of which was tested using the randomization procedure implemented in FSTAT v.2.9.3 (Goudet 1995). The distributions of allele frequencies (‘mode shift indicator’, estimated using the BOTTLENECK software; Cornuet & Luikart 1996) were examined in order to assess deviation from the mutation-drift equilibrium, which can potentially confound inferences drawn from F_{ST}–Q_{ST} comparisons (Merilä & Crnokrak 2001). The amount of differentiation of allele frequencies between each pair of populations was quantified using Weir and Cockerham’s (Weir & Cockerham 1984) standardized F_{ST} and Goodman’s (Goodman 1997) R_{ST}, the latter of which assumes that loci evolve according to the stepwise mutation model (SMM). The dinucleotide loci RtμH and RtSBO3 were excluded from the data set for R_{ST} calculations due to the common occurrence of 1 bp size differences (Appendix 1), suggesting that an SMM-based mutational model was not appropriate for these loci. The statistical significance of the F-values was determined by bootstrapping (10 000 replicates). As the global F_{ST} and R_{ST}

values were very similar (see Results), only F_{ST} was used in further analyses.

Quantitative genetic data

Tadpoles used in the common garden experiments were obtained from laboratory crosses of adults collected from spawning sites at the onset of the breeding season, except for the Ammarnäs population, for which eight freshly-laid spawn clumps were collected from the wild (see below). These procedures ensured that all tadpoles were of the same age at the start of the experiment and subjected to the same early environmental effects, and allowed controlled crosses according to a North Carolina II design (e.g. Lynch & Walsh 1998) to be made, enabling estimation of within- and among-population components of genetic variation in larval life history traits. In all but the Umeå and Ammarnäs (see above) populations, 16 maternal half-sib families (i.e. 32 full-sib families) were created where eggs from each of eight females were fertilized by sperm from four of the 16 males. The Umeå tadpoles stem from 32 maternal half-sib families (16 females and 32 males used). Due to the large difference in the onset of spawning among the populations (Merilä *et al*. 2000b), the starting dates for the experiment also differed. In the case of the southernmost population (Lund), the fertilizations were performed on 9 April 1998, whereas in the case of the northernmost population (Kilpisjärvi), the corresponding date was 4 June 1998. However, the rearing conditions were identical for all populations (see below).

The crosses were carried out following the principles outlined in Laugen *et al*. (2002). The eggs were divided into three different temperature treatments (14, 18 and 22 °C ± 1 °C, two bowls per cross in each temperature) at which they were kept until hatching. Water was changed every third day during embryonic development. When most of the embryos in a given temperature treatment had reached Gosner stage 25 (Gosner 1960), eight randomly-chosen tadpoles from each cross were placed individually in 0.9 L opaque plastic containers at each of two food levels (restricted and ad libitum). This procedure was repeated for each population in the three temperature treatments, resulting in 48 experimental tadpoles per cross. However, due to mortality during the experiment, the final number of tadpoles per family was typically fewer than 48. Every seventh day, the tadpoles were fed a finely-ground 1 : 3 mixture of fish flakes (TetraMin, Ulrich Baensch GmbH, Germany) and rodent pellets (AB Joh. Hansson, Uppsala, Sweden). The amount of food given to each tadpole was 15 mg (restricted) and 45 mg (ad libitum) for the first week, 30 and 90 mg for the second week, and 60 and 180 mg per week thereafter until metamorphosis. The ad libitum level was selected to be such that the individuals did not consume all the food before the next feeding event at any of the temperature treatments. In the restricted food treatment, the tadpoles at the two highest temperature treatments consumed all of their food resources before the next feeding, indicating food limitation, but in the low temperature treatment, the tadpoles frequently had food left even after 7 days of feeding. The tadpoles were raised in dechlorinated tap water that was aerated and aged for at least 24 h before use. The water was changed every seventh day in conjunction with feeding. The light rhythm was 16L : 8D. Close to metamorphosis, the tadpoles were checked every day, and individuals that had reached Gosner stage 42 were noted. Developmental rate, or the age at metamorphosis, was defined as the number of days elapsed between reaching Gosner stages 25 and 42. Size at metamorphosis was defined as the fresh weight of the metamorphs (first rolled gently in paper towel to remove extra water) to the nearest milligram. Comparison of the fresh and dry weights in two of the populations had shown that the two are strongly correlated ($r_{750} = 0.77$, $P < 0.001$ and $r_{795} = 0.81$, $P < 0.001$; J. Merilä *et al*., unpublished). Growth rate was estimated as the weight at metamorphosis corrected for the time (in days) elapsed between Gosner stages 25 and 42. In effect, this estimates the weight the tadpoles would have obtained had they all reached metamorphosis on the same date (the average date of metamorphosis). This is more flexible than dividing the weight by time, which carries a strong assumption that growth is constant over the whole period of growth.

Statistical analyses

Separate analyses were carried out on the weight of a tadpole at metamorphosis, the time taken to reach metamorphosis, and growth rate. For all analyses, an linear model (Lynch & Walsh 1998) was fitted to the data. The response (i.e. weight or time) was assumed to follow a normal distribution. A separate model was fitted for all treatments, so the following model will apply to each treatment t (i.e. temperature–food combination):

$$y_{i,t} \sim N(\eta_{i,t}, \sigma^2_{w,t}) \quad (1)$$

The mean, η_i (i.e. the predicated value for each individual), depends on genetic and environmental factors:

$$\eta_{i,t} = \mu_{p,t} + \phi_{m,t} + \alpha_{f,t} + \varepsilon_{m,f,p,t} \quad (2)$$

Where $\mu_{p,t}$ is the population mean, $\phi_{m,t}$ is the contribution of the mth male to its offspring, $\alpha_{f,t}$ is the contribution of the fth female to its offspring, $\varepsilon_{m,f,t}$ is the interaction between the mth male and the fth female. Growth rate was modelled by including time to metamorphosis in the weight model as a population-specific covariate. μ_p, ϕ_m, α_f and $\varepsilon_{m,f}$ are all modelled as random effects:

$$\begin{aligned} \mu_{p,t} &\sim N(\mu_{0,t}, \sigma^2_{p,t}) \\ \phi_{m,t} &\sim N(0, \sigma^2_{S,t}) \\ \alpha_{f,t} &\sim N(0, \sigma^2_{D,t}) \\ \varepsilon_{m,f,t} &\sim N(0, \sigma^2_{I,t}) \end{aligned} \tag{3}$$

These experimental variance components (except for $\mu_{p,t}$) were assumed to be constant across the populations, but were estimated separately for the different treatments. Although there were slight differences in the size of the variance components in different traits for different populations and treatments (results not shown), this assumption was justified as these variations were small relative to the among-population components of variance for each trait. Hence, their effect on the Q_{ST} estimates was negligible. Accordingly, as the main aim of our analysis was to estimate Q_{ST}, the assumption about constant within-population experimental variance components across the populations simplified the analysis and presentation of the results considerably. For instance, although only broad-sense estimates of additive genetic variance (V_A) were available for the Ammarnäs population (full-sib data; see above), assuming a constant V_A across populations allowed us to use narrow sense estimates of V_A from other populations for Ammarnäs as well (note: estimated broad-sense V_As were very similar to the narrow-sense estimates for other populations). The random effects were then modelled as being sums of the underlying quantitative genetic variances:

$$\begin{aligned} \sigma^2_{p,t} &= V_{GB,t} \\ \sigma^2_{S,t} &= V_{GW,t}/4 \\ \sigma^2_{D,t} &= V_{D,t}/4 + V_{M,t} \\ \sigma^2_{I,t} &= V_{D,t}/4 \\ \sigma^2_{W,t} &= V_{GW,t}/2 + 3V_{D,t}/4 + V_{Ew,t} \end{aligned} \tag{4}$$

Where $V_{GB,t}$, $V_{GW,t}$, $V_{D,t}$, $V_{M,t}$ and $V_{Ew,t}$ are the treatment specific population, additive, dominance, maternal and within environmental effects.

The models were fitted using a Bayesian approach (e.g. Gelman *et al*. 1995), which meant that the quantitative genetic components could be estimated directly, rather than taking the traditional approach of estimating the experimental variance components and then calculating the quantitative genetic variances by subtraction. The advantage of our approach is that the variances are all restricted to be positive, and also the full posterior distribution of the parameters (i.e. the distribution after the information from the data has been included in the model) is estimated.

Prior distributions were set to be fairly flat, representing little knowledge. Specifically, μ_p was given an N(0, 10^3) prior. The inverses of all of the variances were given exponentially-distributed priors with mean 1, except for the inverse of population variances, which were given exponentially-distributed priors with mean 2. This meant that the prior distribution of Q_{ST} was flat. The model was fitted using a Gibbs sampler with the WINBUGS package (Spiegelhalter *et al*. 1999). For each analysis, two chains were run after a burn-in of 10 000 iterations; every five of the next 25 000 iterations were taken to give a total of 2×5000 draws from the posterior distribution.

Statistics based on the model were calculated for each iteration drawn from the posterior, so that the distribution of the statistic over the iterations is correct and includes the effect of any correlation between parameters. To provide informal posterior distributions for F_{ST} (both overall and pairwise F_{ST}), 10 000 values were drawn from a normal distribution with means and variance identical to those estimated for the F_{ST} statistic. Note that the pairwise F_{ST}s are taken as being independent.

Q_{ST} was calculated as (Prout & Barker 1989; Merilä & Crnokrak 2001):

$$Q_{ST} = \frac{V_{GB}}{2V_{GW} + V_{GB}} \tag{5}$$

and pairwise Q_{ST}s were calculated using the same formula, but with V_{GB} being calculated as the variance of the estimates of the population means of the two populations.

Correlations between pairwise estimates of Q_{ST}, F_{ST} and geographical distance were calculated from the posterior distributions, using the odds (i.e. $p/(1-p)$; Rousset 1997) of Q_{ST} and F_{ST}. These are equivalent to the correlations that are calculated in Mantel tests, but because we use the full posterior distribution of the parameters, the confidence limits of the correlation coefficients take the correlations between pair-wise Q_{ST} estimates into account.

Many of the results are expressed as the posterior mode and 95% HPD (Highest Posterior Density) confidence intervals. The posterior mode is comparable to the maximum likelihood estimate. Ninety-five percent HPD confidence intervals are calculated so that they contain 95% of the posterior probability mass, and all of the points within the interval have higher probability densities than the points outside (Gelman *et al*. 1995).

Results

Molecular genetic variability

The average number of individuals successfully genotyped for each locus varied from 30.4 to 39.9 between populations (mean 35.2; Table 1). Altogether, 108 allelic variants were found, 33 of which were unique for a single population. After correcting for multiple tests using the Bonferroni procedure (Rice 1987), the genotype frequencies did not deviate significantly from expected Hardy–Weinberg proportions in any of the six populations (Table 1). Allele

© 2003 Blackwell Publishing Ltd,

Table 2 Pairwise estimates of F_{ST} (95% C.I.s) among the six common frog populations

	Uppsala	Umeå	Ammarnäs	Kiruna	Kilpisjärvi
Lund	0.158 (0.088–0.264)	0.167 (0.110–0.218)	0.219 (0.140–0.309)	0.207 (0127–0.301)	0.260 (0.169–0.356)
Uppsala	—	0.245 (0.117–0.415)	0.241 (0.191–0.292)	0.228 (0.143–0.347)	0.363 (0.272–0.461)
Umeå		—	0.238 (0.125–0.378)	0.130 (0.077–0.192)	0.259 (0.122–0.429)
Ammarnäs			—	0.211 (0.136–0.318)	0.330 (0.179–0.482)
Kiruna				—	0.165 (0.099–0.234)

frequency distributions (see Appendix 1) in all of the populations were L-shaped, as expected for populations in mutation-drift equilibrium.

The average gene diversity estimates for the populations varied from 0.504 to 0.667 (Table 1). The southernmost Tvedöra population harboured 18 of the 33 unique alleles and was also the most variable in terms of allelic and gene diversities. There was a nonsignificant tendency for allelic richness to decrease with increasing latitude ($r_s = -0.714$, $P = 0.087$), but no correlation between gene diversity and latitude ($r_s = -0.086$, $P = 0.869$)

Among-population differentiation at the marker loci was substantial, the overall F_{ST} being 0.235 (95% HPDI: 0.182–0.300) and the overall R_{ST} being 0.243 (95% CI: 0.212–0.292). In view of the similarity between the F_{ST} and R_{ST} estimates, only F_{ST} were used in further analyses. Pairwise estimates varied from 0.130 to 0.363 (Table 2), and the degree of differentiation tended to increase with increasing geographical distance between the populations as revealed by a positive correlation between the pairwise F_{ST} values and geographical distances ($r = 0.20$, 95% HPDI: 0.132–0.281).

Quantitative genetic variability

For all of the traits, there was substantial heterogeneity in the population mean values that could be attributed to both treatment and population specific effects (Fig. 2a–c; Table 3). The mean age at metamorphosis was negatively linearly correlated with latitude in all treatments (Fig. 2d–f; Laugen *et al*. 2003a), whereas the effects of latitude and treatment were more heterogeneous for size and growth rate (Fig. 2). Nevertheless, even within each of the treatment combinations, the population mean values displayed significant heterogeneity (Fig. 2), as also evidenced by significant (absolute) among-population components of variance (Table 3). These, in combination with low, but still significant, additive genetic components of variation in each trait (Table 3), translated to high Q_{ST} estimates in all of the traits and the majority of treatment combinations (Table 3; Fig. 3). Thus, the overall quantitative genetic divergence ($Q_{ST} \approx 0.81$) clearly exceeded that in neutral marker loci in all traits and treatment combinations (Fig. 3). Apart from two cases (age at metamorphosis), the differences were also statistically significant (Fig. 3).

The degree of quantitative genetic differentiation in age at metamorphosis between different populations was positively correlated with the geographical distance separating the populations (Fig. 4a). For the other two traits, there was no consistent relationship between quantitative genetic differentiation and geographical distance (Fig. 4b,c). The degree of among-population differentiation in quantitative traits was not consistently predictable from the degree of differentiation in molecular markers (Fig. 4d–f). In the case of age at metamorphosis, four out of the six comparisons revealed a positive correlation between F_{ST} and Q_{ST} estimates (Fig. 4d), whereas for metamorphic size, one significantly positive and two significantly negative relationships were observed (Fig. 4e). In the case of growth rate, only one of the correlations (positive) was significant (Fig. 4f). These findings clearly demonstrate that genotype–environment interactions can mask or enhance the correspondence between quantitative and molecular genetic measures of differentiation.

Finally, it is also worth noting that in all treatments, the correlation between quantitative genetic differentiation in metamorphic age and geographical distance was significantly higher than that between molecular genetic differentiation and geographical distance (Fig. 4a). This further reinforces the conclusion that the clinal differentiation in age at metamorphosis has been driven by clinally varying natural selection.

Discussion

The most salient findings of this study were the very high degree of genetic population subdivision among the relatively young common frog populations, and the

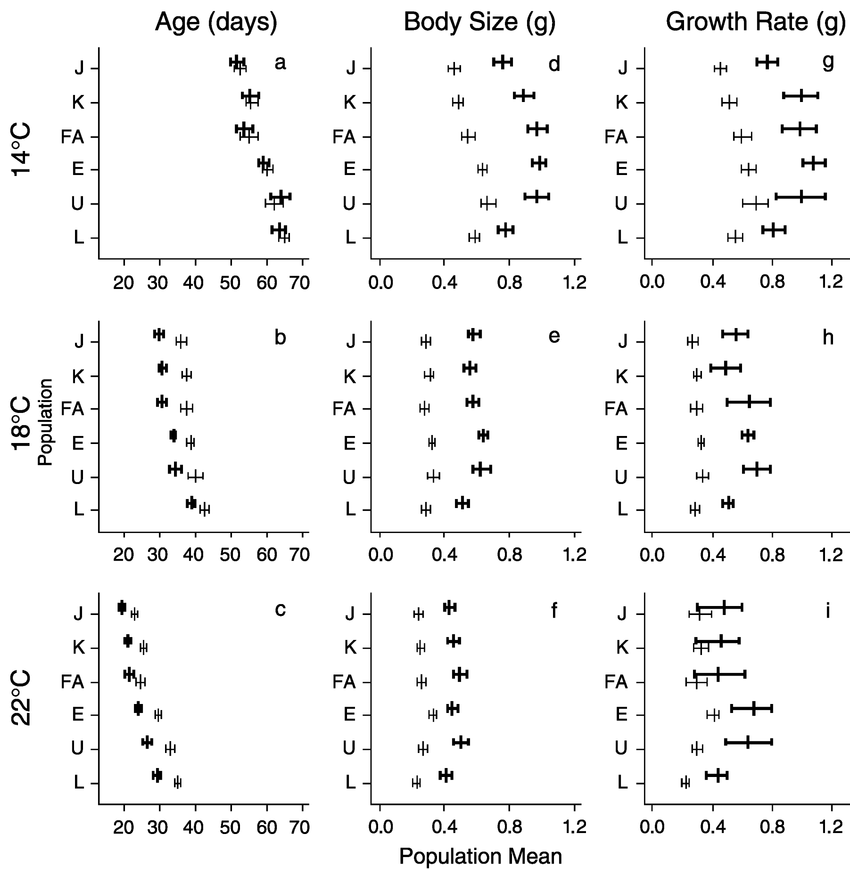

Fig. 2 Mean (± 95% HPD) (a–c) age, (d–f) body size and (g–i) growth rate (i.e. age corrected body size) in different populations and treatment conditions. Populations are arranged in descending order from north to south (J = Kilpisjärvi, K = Kiruna, FA = Ammarnäs, E = Umeå, U = Uppsala, L = Lund). Bold symbols = high food treatment; normal symbols = low food treatment.

Table 3 Among- and within-(= additive genetic) population variance components with corresponding Q_{ST} estimates (± 95% HPDs) in different treatments for different larval traits in common frogs

	14 °C		18 °C		22 °C	
	High	Low	High	Low	High	Low
(a) Age at metamorphosis						
Among	13.57	15.92	3.49	7.41	15.36	8.47
	(3.70–57.10)	(4.18–65.85)	(0.61–15.85)	(2.10–30.40)	(4.13–58.76)	(2.50–34.32)
Within	0.86	1.21	3.80	0.96	0.60	0.71
	(0.44–10.31)	(0–17.61)	(0.12–7.92)	(0.14–3.67)	(0.12–2.47)	(0.09–3.48)
Q_{ST}	0.93	0.93	0.30	0.85	0.96	0.89
	(0.41–1)	(0.29–1)	(0.11–0.85)	(0.49–0.99)	(0.75–1)	(0.55–0.99)
(b) Weight at metamorphosis						
Among	0.88	1.08	0.51	0.59	0.55	0.50
	(0.26–3.48)	(0.28–4.37)	(0.14–2.11)	(0.19–2.39)	(0.18–2.14)	(0.18–2.21)
Within	0.16	0.31	0.09	0.14	0.07	0.15
	(0.09–0.27)	(0.13–0.64)	(0.06–0.14)	(0.08–0.25)	(0.05–0.11)	(0.08–0.26)
Q_{ST}	0.81	0.73	0.81	0.73	0.88	0.73
	(0.57–0.95)	(0.42–0.93)	(0.58–0.95)	(0.50–0.93)	(0.66–0.96)	(0.46–0.92)
(c) Growth rate to metamorphosis						
Among	0.87	1.40	0.51	0.91	0.66	1.28
	(0.25–3.71)	(0.37–6.03)	(0.17–2.02)	(0.22–4.09)	(0.21–2.78)	(0.27–6.00)
Within	0.16	0.29	0.09	0.14	0.07	0.14
	(0.09–0.28)	(0.13–0.63)	(0.06–0.13)	(0.08–0.25)	(0.05–0.10)	(0.08–0.25)
Q_{ST}	0.83	0.78	0.80	0.84	0.90	0.90
	(0.57–0.96)	(0.49–0.96)	(0.60–0.95)	(0.60–0.97)	(0.73–0.98)	(0.67–0.98)

Low = low food level; High = high food level.

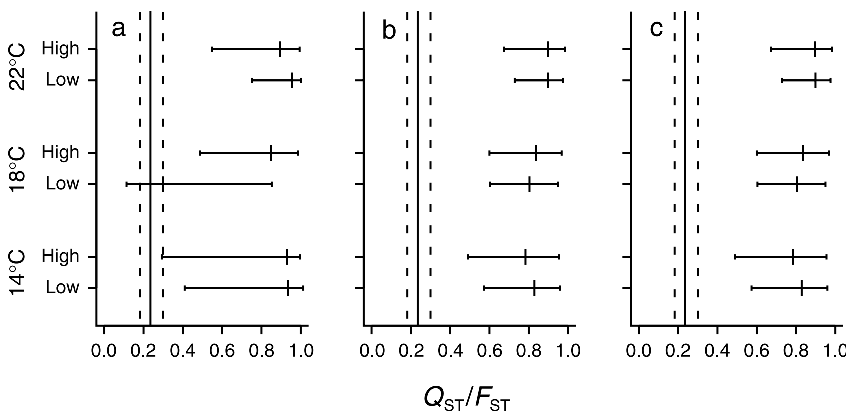

Fig. 3 Comparison of overall Q_{ST} and F_{ST} estimates for (a) age, (b) body size and (c) growth rate (i.e. age corrected body size) under different treatment conditions. The vertical line depicts F_{ST} (± 95% C.I. — vertical dotted lines) and vertical bars Q_{ST} (± 95% HPDs — horizontal lines); 'low' and 'high' refer to low and high food level treatments.

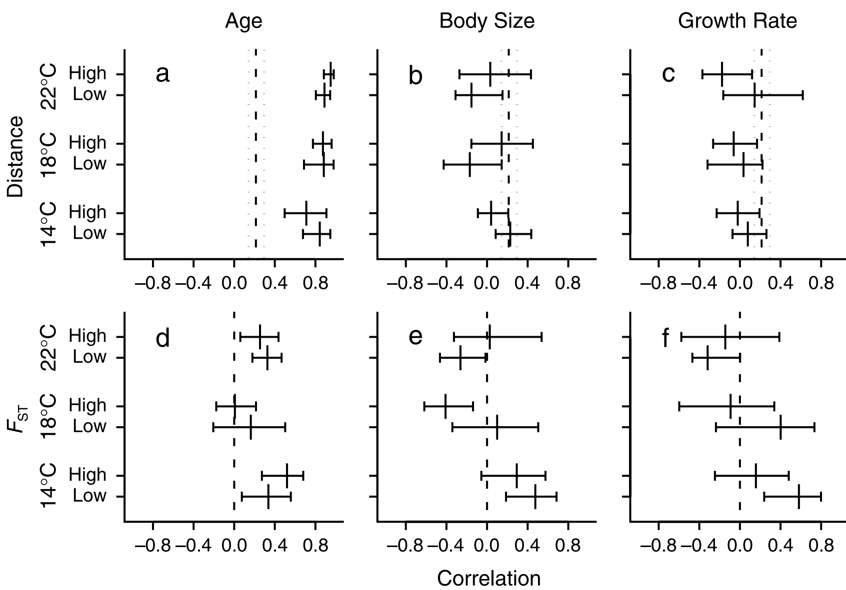

Fig. 4 Posterior modes (± 95% HPDs) for correlations of pairwise comparisons of (a–c) $Q_{ST}/(1-Q_{ST})$ and geographical distance (d–f) $Q_{ST}/(1-Q_{ST})$ and $F_{ST}/(1-F_{ST})$. In (a–c), the dashed line is mode of correlation between geographical distance and $F_{ST}/(1-F_{ST})$; dotted lines: 95% HPDs. In (d–f), the dashed line depicts a zero correlation.

substantially higher degree of population differentiation in quantitative traits as compared with neutral marker loci. Furthermore, we only found weak evidence for the contention that the degree of differentiation in quantitative traits is predictable from the degree of differentiation in neutral marker genes. Instead, our results suggest that the Q_{ST} estimates may be sensitive to genotype–environment interactions. In particular, although the environmental influences on the expression of Q_{ST} estimates were relatively weak compared with overall patterns of differentiation across different populations and treatments, the pairwise correlations between F_{ST} and Q_{ST} estimates among different populations were sensitive to conditions under which quantitative trait variability had been measured. In the following section, these findings and their implications, as well as some of the assumptions of our analyses, are discussed in detail.

Despite the ample opportunity for differentiation due to genetic drift, as shown by the high degree of differentiation in the neutral marker genes, two lines of evidence indicate that natural selection has been a more important evolutionary force than drift in driving the divergence of quantitative traits among common frog populations. First, the degree of divergence in all three quantitative traits (Q_{ST}) exceeded that in neutral markers (F_{ST}), a pattern that has also been observed in the majority of similar comparisons in other taxa (Merilä & Crnokrak 2001; McKay & Latta 2002; but see Lee & Frost 2002). In fact, the difference between F_{ST} and Q_{ST} estimates in this study ($Q_{ST} - F_{ST}$ = 0.58) greatly exceeds that in any of the studies listed in Merilä & Crnokrak (2001), where the average difference was 0.16 (maximum = 0.49; Bonnin *et al.* 1996). This may be partly due to the fact that, unlike previous studies (but see Koskinen *et al.* 2002), we estimated Q_{ST} from a half-sib design and as a consequence, Q_{ST} was not underestimated, as would be the case for full-sib designs when maternal or dominance effects are present (Merilä & Crnokrak 2001). Secondly, in the case of age at metamorphosis, which was

the only trait showing a clear pattern of latitudinally-ordered differentiation, the correlation between pairwise Q_{ST} estimates and geographical distances separating populations exceeded that between F_{ST} estimates and geographical distances. This provides strong evidence for the interpretation that the latitudinal cline in this trait is driven by clinally-varying natural selection, most likely because faster metamorphosis in the north is favoured because of the shortening season towards the north (Laugen *et al*. 2003a). It should be noted that if the latitudinal cline in metamorphic age should be mainly driven by neutral divergence under the isolation by distance scenario, we would have expected concordant patterns of isolation by distance for both F_{ST} and Q_{ST}. Using a similar, but slightly different approach, Gockel *et al*. (2001) inferred that a latitudinal body size cline in Australian *Drosophila melanogaster* is selection driven. However, as far as we are aware, the only other studies where molecular markers have been used to infer the relative importance of natural selection and genetic drift as determinants of clinal variation are those by Merilä (1997) and Storz (2002). However, the 'Q_{ST}' estimates in the latter two studies were based on measurements of wild-caught animals and hence, they might have overestimated the degree of genetic differentiation in quantitative traits due to environmental effects. Comparing Q_{ST} estimates derived from common garden vs. wild-collected data in a copepod, *Eurytrmora affinis*, Lee & Frost (2002) found the latter to be 1.8 times higher than the former, suggesting a role for environmental effects inflating the Q_{ST} estimates in the wild. Returning to the results of the present study, we note that as in the case of most other studies of geographical variation, direct estimates of natural selection on metamorphic age in different populations are lacking. However, indirect evidence (Laugen *et al*. 2003a; see also Merilä *et al*. 2000a; Laugen *et al*. 2002; Laurila *et al*. 2002) suggests that faster development of northern compared with southern frogs reflects adaptation to the significantly shorter growth season in the north (about 98 days) compared with the south (about 217 days). The reason why size and growth rate did not conform to this same latitudinally-ordered pattern of differentiation cannot be answered in this study, but an obvious possibility is that factors other than seasonal time constraints are more important determinants of divergence in these traits than in age at metamorphosis.

In a recent review, Merilä & Crnokrak (2001; see also Lynch *et al*. 1999; Latta & McKay 2002) found that the degree of among-population differentiation in quantitative traits was predictable from knowledge of the degree of differentiation in neutral markers across the different studies conducted so far. Although this suggests that molecular markers could be used as surrogate estimates of the degree of adaptive differentiation when quantitative genetic data are not available, the conclusion may be premature as the data on which this result is based comprised studies where both the F_{ST} and the Q_{ST} values ranged from zero to unity. Such variation is seldom observed in intraspecific studies and in fact, only two studies to date have attempted to test for this relationship with intraspecific data (Morgan *et al*. 2001; Steinger *et al*. 2002; see also Long & Singh 1995). In contrast to the results of Morgan *et al*. (2001) and Steinger *et al*. (2002), who found evidence for a positive correlation between pairwise F_{ST} and Q_{ST} estimates, we found only weak and inconsistent evidence for such a correlation in the present study. In fact, both negative and positive correlations were found for one of the traits depending on the treatment conditions considered. This suggests that knowledge of the degree of population differentiation in molecular markers in *R. temporaria* is not informative about the degree of genetic differentiation in ecologically-important traits, at least not over the geographical scale considered here.

Although genotype by environment interactions are widespread, and quantitative genetic parameters are known to be sensitive to the environmental conditions under which they are estimated (Hoffmann & Merilä 1999; Hoffmann & Hercus 2000), little attention has been paid to the possibility that Q_{ST} estimates might also be sensitive to the conditions under which they are measured (Merilä & Crnokrak 2001). In fact, both under- and overestimation of actual genetic differences among populations are possible, as illustrated by the widespread occurrence of countergradient variation (Conover & Schultz 1995). In the present study, the overall estimates of Q_{ST} were relatively robust, indicating similar levels of differentiation for all traits across the six different treatment conditions (cf. Fig. 3). However, the confidence intervals for the estimates were broad, and the pairwise comparisons of F_{ST} and Q_{ST} estimates displayed a high degree of environment dependency (cf. Fig. 4e,f). Consequently, in accordance with the findings of Lee & Frost (2002), the results caution against uncritical use of Q_{ST} estimates derived from purely phenotypic data, and provide an example of how interactions between genetic and environmental factors can complicate evolutionary inferences.

Although our breeding design allowed statistical control over the potentially confounding effects of maternal and dominance contributions to within-population estimates of genetic variance, one potential source of uncertainty in our analyses resides in uncontrolled cross-generation ('grandparent effect') maternal or environmental effects (e.g. Roff 1997), which might have inflated our Q_{ST} estimates. Maternal effects transmitted through eggs are known to have substantial effects on offspring performance in many taxa (reviews in Rossiter 1996; Mousseau & Fox 1998), including frogs (Kaplan 1998). However, two lines of evidence suggest that maternal effects are unlikely to exert any substantial bias on our estimates of population

© 2003 Blackwell Publishing Ltd,

differentiation in the traits studied. First, although egg size effects are thought to be the main pathway for expression of maternal effects in amphibians (Kaplan 1998), they have been shown to have only a minor impact on age and size at metamorphosis along the latitudinal gradient studied here (Laurila *et al*. 2002; Laugen *et al*. 2003a). Second, hybridization experiments between frogs from Lund and Umeå have demonstrated that although part of the among-population differences in metamorphic traits between the two populations may be explained by maternal effects other than egg size (whether genetic or environmental), these seem to be small compared with additive genetic effects (Laugen *et al*. 2002). Consequently, it seems likely that the very high Q_{ST} estimates reflect a truly high degree of genetic differentiation among the study populations. Note also that the average Q_{ST}-value from our study (0.81) exceeds that obtained from the majority of other studies (average $Q_{ST} = 0.37$; Merilä & Crnokrak 2001), and is similar to that obtained in studies of *Arabidobsis thaliana* (Kuittinen *et al*. 1997: $Q_{ST} = 0.88$) and *Arabis fecunda* (McKay *et al*. 2001: $Q_{ST} = 0.94$).

Our analysis of variability in presumably neutral microsatellite loci revealed a very high degree of genetic subdivision among the relatively young Scandinavian common frog populations. The colonization of the Scandinavian peninsula by frogs, as with many other organisms, was not possible until the retreat of the glacial ice sheet *c*. 10 000 years ago (e.g. Taberlet *et al*. 1998). This implies that the time drift has had to act on the populations is rather short, probably less than 2000 generations (assuming a 4 year generation interval). Given this, the observed levels of neutral genetic differentiation among the populations studied appear high, and argue against the view that boreal-temperate zone organisms would generally be genetically more homogenous than organisms at lower latitudes (Hamrick & Godt 1990; but see Pamilo & Savolainen 1999). A high degree of population subdivision is indeed typical for amphibians (Ward *et al*. 1992), even over geographical scales much smaller than that examined in our study (Newman & Squire 2001). Even though our F_{ST} estimates are high, they may still be downward biased (e.g. Hedrick 1999; Balloux *et al*. 2000) because of the high mutation rates of microsatellite loci (Ellegren 2000). Under an island model of population structure in mutation-drift equilibrium, with mutational dynamics conforming to an infinite alleles model, the expected degree of population differentiation is given by (Wright 1969): $F_{ST} = (1 + 4N_e(m + \mu))^{-1}$, where N_e = effective population size, m = migration rate, μ = mutation rate. Using the average $F_{ST} = 0.24$ in our study, we calculate $N_e(m + \mu) = 0.84$. Using an N_e of 141 for the most northern population, estimated from capture-recapture data (J. Merilä, unpublished), using equation 2.27 of Hartl & Clark (1989), a mutation rate of 0.006 would be required to achieve the observed level of differentiation (under the assumption of no migration). This is six times larger than the typical mutation rate estimates for microsatellite loci ($1 \times 10^{-3} - 1 \times 10^{-4}$ mutations per gamete per generation; Ellegren 2000), but some of this difference can be made up by local migration from nearby populations. Furthermore, it is likely that our N_e estimate is conservative (the northernmost population is the smallest population in our study) and hence, the discrepancy is probably even less. Even if we allowed overestimation of F_{ST} by a factor of two [i.e. true $F_{ST} = 0.46$ instead of 0.23 (as it would be if $\mu = 0.001$, $m = 0$ and $N_e = 300$)], the Q_{ST} estimates in Fig. 3 would still be significantly higher than the F_{ST}. Consequently, our inference that $Q_{ST} > F_{ST}$ for all traits in most environments is not sensitive even to rather large underestimation of F_{ST}.

Finally, we note that interglacial expansions from the refugial areas south of the ice (and permafrost) have usually been rapid 'leading-edge invasions', resulting in reduced intraspecific diversity due to associated repeated founder effects (Hewitt 2000). A number of studies have reported negative correlations between genetic diversity and latitude in postglacial colonizers, giving evidence for a sequential loss of diversity during a range expansion (e.g. Merilä *et al*. 1996; Alexandrino *et al*. 2000; Demboski & Cook 2001). The fact that we found weak or no evidence for a latitudinal trend in neutral genetic diversity suggests that common frogs have not been subjected to sequential bottlenecks during the colonization of Scandinavia. However, since the colonization of Sweden by the common frog may have proceeded either from the south, north or from both directions (see Taberlet *et al*. 1998; Hewitt 2000), further studies including potential ancestral populations and other markers are needed to assess to which, if any, direction(s) the genetic diversity is expected co-vary with latitude.

In conclusion, the results of this study demonstrate a high degree of differentiation among relatively young common frog populations in both (presumably) neutral marker genes and genes coding quantitative traits. Differentiation in the latter greatly exceeded that in the former, suggesting that the differentiation in quantitative traits has been driven by directional natural selection favouring faster developing genotypes in the north as compared to the south. This inference was further reinforced by the observation that the correlation between geographical distance and the Q_{ST} for metamorphic age was much stronger than the correlation between geographical distance and F_{ST}. However, although the degree of genetic differentiation in metamorphic size and growth rate also exceeds that in neutral marker genes, this differentiation was not latitudinally ordered. Hence, divergence in these traits might be driven by selection arising from factors different to that/those on age at metamorphosis.

© 2003 Blackwell Publishing Ltd, *Molecular Ecology*, 10.1046/j.1365-294X.2003.01865.x

Acknowledgements

Ingemar Jönsson, Oula Kalttopää, Satu Karttunen, Eevi Karvonen, Niclas Kolm, Katja Räsänen and Fredrik Söderman helped with obtaining the frogs and rearing the tadpoles, whereas Sofia Berlin, Joost van Heerdwarden, Markus Johansson, David Lesbarreres and Leena Laaksonen helped with microsatellite work. Thanks are also due to three anonymous referees for their constructive suggestions on an earlier version of this paper. This study was supported by the Swedish Natural Science Research Council and the Academy of Finland.

References

Alexandrino J, Froufe E, Arntzen JW, Ferrand N (2000) Genetic subdivision, glacial refugia and postglacial recolonization in the golden-striped salamander, *Chioglossa lusitanica* (Amphibia: Urodela). *Molecular Ecology*, **9**, 771–781.

Balloux F, Brünner H, Lugon-Moulin N, Hausser J, Goudet J (2000) Microsatellites can be misleading: an empirical and simulation study. *Evolution*, **54**, 1414–1422.

Berlin S, Merilä J, Ellegren H (2000) Isolation and characterisation of polymorphic microsatellite loci in the common frog *Rana temporaria*. *Molecular Ecology*, **9**, 1938–1939.

Bonnin I, Prosperi J-M, Olivier I (1996) Genetic markers and quantitative genetic variation in *Medicago truncatula* (Leguminosae): a comparative analysis of population structure. *Genetics*, **143**, 1795–1805.

Bruford MW, Hanotte O, Brookfield JFY, Burke T (1992) Single-locus and multilocus DNA fingerprinting. In: *Molecular Genetic Analysis of Populations. A Practical Approach* (ed. Hoelzel AR), pp. 225–269. Oxford University Press, Oxford.

Conover DO, Schultz ET (1995) Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution*, **10**, 248–252.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Crnokrak P, Merilä J (2002) Genetic population divergence: markers and traits. *Trends in Ecology and Evolution*, **17**, 501.

Demboski JR, Cook JA (2001) Phylogeography of the dusky shrew, *Sorex monticolus* (Insectivora, Soricidae): insight into deep and shallow history in northwestern North America. *Molecular Ecology*, **10**, 1227–1240.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.)] endemic to Morocco. *Theoretical and Applied Genetics*, **92**, 832–839.

Ellegren H (2000) Microsatellite mutations in the germline: implications for evolutionary inference. *Trends in Genetics*, **16**, 551–558.

Endler JA (1977) *Geographic Variation, Speciation, and Clines*. Princeton University Press, Princeton.

Fog K, Schmedes A, de Lasson DR (1997) *Nordens Padder Og Krybdyr*. G.E.C. Gad, Copenhagen.

Fraser DL, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology*, **10**, 2741–2752.

Gelman AJ, Carlin JB, Stern HS, Rubin D (1995) *Bayesian Data Analysis*. Chapman & Hall, London.

Gilchrist AS, Partridge L (1999) A comparison of the genetic basis of wing size divergence in three parallel body size clines of *Drosophila melanogaster*. *Genetics*, **153**, 1775–1787.

Gockel J, Kennigton WJ, Hoffmann A, Golstein DB, Partridge L (2001) Nonclinality of molecular variation implicates selection on maintaining a morphological cline of *Drosophila melanogaster*. *Genetics*, **158**, 319–323.

Goodman SJ (1997) Rst Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. *Molecular Ecology*, **6**, 881–885.

Gosner KL (1960) A simplified table for staging anuran embryos and larvae. *Herpetologica*, **16**, 183–190.

Goudet J (1995) FSTAT (vers. 1.2), a computer program to calculate F-statistics. *Journal of Heredity*, **86**, 485–486.

Haig SM (1998) Molecular contributions to conservation. *Ecology*, **79**, 413–425.

Hamrick JL, Godt MJ (1990) Allozyme diversity in plant species. In: *Plant Population Genetics, Breeding and Genetic Resources* (eds Brown ADH, Clegg MT, Kahler AL, Weir BS), pp. 43–63. Sinauer, Sunderland, MA.

Hartl DL, Clark AG (1989) *Principles of Population Genetics*. Sinauer Associates, Sunderland, MA.

Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.

Hewitt G (2000) The genetic legacy of quaternary ice ages. *Nature*, **405**, 907–913.

Hoffmann AA, Hercus MJ (2000) Environmental stress as an evolutionary force. *Bioscience*, **50**, 217–226.

Hoffmann AA, Merilä J (1999) Heritable variation and evolution under favourable and unfavourable conditions. *Trends in Ecology and Evolution*, **14**, 96–101.

Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science*, **287**, 308–309.

Kaplan RH (1998) Maternal effects, developmental plasticity, and life history evolution: an amphibian model. In: *Maternal Effects as Adaptations* (eds Mousseau TA, Fox CW), pp. 244–260. Oxford University Press, New York.

Koskinen MO, Haugen TO, Primmer CR (2002) Contemporary fisherian life-history evolution in small salmonid populations. *Nature*, **419**, 826–830.

Kuittinen H, Mattila A, Savolainen O (1997) Genetic variation at marker loci and in quantitative traits in natural populations of *Arabidopsis thaliana*. *Heredity*, **79**, 144–152.

Latta RG, McKay JK (2002) Genetic population divergence: markers and traits — Response. *Trends in Ecology and Evolution*, **17**, 501–502.

Laugen TA, Laurila L, Merilä J (2002) Maternal and genetic contributions to geographical variation in *Rana temporaria* larval life histories. *Biological Journal of the Linnean Society*, **76**, 61–70.

Laugen AT, Laurila L, Merilä J (2003b) Latitudinal variation and temperature-dependency of embryonic development and growth in *Rana temporaria*. *Oecologia*, in press.

Laugen AT, Laurila A, Räsänen K, Merilä J (2003a) Latitudinal countergradient variation in the common frog (*Rana temporaria*) development rates — evidence for local adaptation. *Journal of Evolutionary Biology*, in press.

Laurila A, Karttunen S, Merilä J (2002) Adaptive phenotypic plasticity and genetics of larval life histories in two *Rana temporaria* populations. *Evolution*, **56**, 617–627.

Lee CE, Frost BW (2002) Morphological stasis in the *Eurytemora affinis* species complex (Copepoda: Temoridae). *Hydrobiologia*, **480**, 111–128.

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics*, **27**, 237–277.

Long AD, Singh RS (1995) Molecules versus morphology: the detection of selection acting on morphological characters along a cline in *Drosophila melanogaster*. *Heredity*, **74**, 569–581.

Lynch M (1996) A quantitative genetic perspective on conservation issues. In: *Conservation Genetics: Case Histories from Nature* (eds Avise J, Hamrick J), pp. 471–501. Chapman & Hall, New York.

Lynch M, Pfrender M, Spitze K *et al.* (1999) The quantitative and molecular genetic architecture of a subdivided species. *Evolution*, **53**, 100–110.

Lynch M, Walsh B (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA.

McKay JK, Bishop JG, Lin J-Z, Richards JH, Sala A, Mitchell-Olds T (2001) Local adaptation across a climatic gradient despite small effective population size in the rare sapphire rockcress. *Proceedings of the Royal Society of London B*, **268**, 1–7.

McKay JM, Latta RG (2002) Adaptive population divergence: markers, QTL and traits. *Trends in Ecology and Evolution*, **17**, 285–291.

Merilä J (1997) Quantitative trait and allozyme divergence in the greenfinch (*Carduelis chloris*, Aves: Fringillidae). *Biological Journal of Linnean Society*, **61**, 243–266.

Merilä J, Björklund M, Baker AJ (1996) Genetic population structure and northward decline of genetic variability in the Greenfinch, *Carduelis chloris*. *Evolution*, **50**, 2548–2557.

Merilä J, Crnokrak P (2001) Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology*, **14**, 892–903.

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

Merilä J, Pahkala M, Johanson U (2000b) A frog perspective on increased ultraviolet-B radiation, climate change and latitudinal adaptation. *Annales Zoologici Fennici*, **37**, 129–134.

Miaud C, Guyétant R, Elmberg J (1999) Variations in life-history traits in the common frog (*Rana temporaria*) (Amphibia: Anura): a literature review and new data from the French Alps. *Journal of Zoology*, **249**, 61–73.

Miaud C, Merilä J (2001) Local adaptation or environmental induction? Causes of population differentiation in alpine amphibians. *Biota*, **2**, 31–50.

Morgan KK, Hicks J, Spitze K *et al.* (2001) Patterns of genetic architecture for life-history traits and molecular markers in a subdivided species. *Evolution*, **55**, 1753–1761.

Moritz C (1994) Uses of molecular phylogenies for conservation. *Philosophical Transactions of the Royal Society of London Series B — Biological Sciences*, **349**, 113–118.

Moritz C, Lavery S, Slade R (1995) Using allele frequency and phylogeny to define units for conservation and management. *American Fisheries Society Symposium*, **17**, 249–262.

Mousseau TA, Fox CW (1998) *Maternal Effects as Adaptations*. Oxford University Press, New York.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Newman RA, Squire T (2001) Mirosatellite variation and fine-scale population structure in the wood frog (*Rana sylvatica*). *Molecular Ecology*, **10**, 1087–1100.

Pahkala M, Laurila A, Merilä J (2002) Effects of ultraviolet radiation on common frog *Rana temporaria* embryos along a latitudinal gradient. *Oecologia*, **133**, 458–465.

Pamilo P, Savolainen O (1999) Post-glacial colonization, drift, local selection and conservation value of population: a northern perspective. *Hereditas*, **130**, 229–238.

Pfender ME, Spitze K, Hicks J, Kendall M, Latta L, Lynch M (2000) Lack of concordance between genetic diversity estimates at the molecular and quantitative-trait levels. *Conservation Genetics*, **1**, 263–269.

Pidancier N, Gauthier P, Miquel C, Pompanon F (2001) Polymorphic microsatellite DNA loci identified in the common frog (*Rana temporaria*, Amphibia, Ranidae). *Molecular Ecology Notes*, **2**, 304–305.

Prout T, Barker JSF (1989) Ecological aspects of the heritability of body size in *Drosophila buzzatii*. *Genetics*, **123**, 803–813.

Reed DH, Frankham R (2001) How closely related are molecular and quantitative measures of genetic variation? A meta-analysis. *Evolution*, **55**, 1095–1103.

Reznick DN, Shaw R, Rodd FH, Shaw RG (1997) Evaluation of the rate of evolution in a natural population of guppies. *Science*, **275**, 1934–1937.

Rice WR (1987) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rieseberg LH, Widmer A, Arntz AM, Burke JM (2002) Directional selection is the primary cause of phenotypic diversification. *Proceedings of the National Academy of Sciences USA*, **99**, 12242–12245.

Roff DA (1997) *Evolutionary Quantitative Genetics*. Chapman & Hall, New York.

Rogers AR (1986) Population differences in quantitative characters as opposed to gene frequencies. *American Naturalist*, **127**, 729–730.

Rossiter M (1996) Incidence and consequences of inherited environmental effects. *Annual Review of Ecology Systematics*, **27**, 451–476.

Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Rowe G, Beebee TJC (2001) Polymerase chain reaction primers for microsatellite loci in the common from *Rana temporaria*. *Molecular Ecology Notes*, **1**, 6–7.

Spiegelhalter DJ, Thomas A, Best NG (1999) *WinBUGS Version 1.2 User Manual*. MRC Biostatistics Unit, Cambridge, UK.

Steinger T, Haldimann K, Leiss A, Müller-Schärer (2002) Does natural selection promote population divergence? A comparative analysis of population structure using amplified fragment length polymorphism markers and quantitative traits. *Molecular Ecology*, **11**, 2583–2590.

Storfer A (1996) Quantitative genetics: a promising approach for the assessment of genetic variation in endangered species. *Trends in Ecology and Evolution*, **11**, 343–348.

Storz JF (2002) Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analysis of clinal variation. *Molecular Ecology*, **11**, 2537–2551.

Taberlet P, Fumagalli L, Cosson AG, Wust S, Andjean F (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–464.

Vos CC, Antonisse-De Jong AG, Goedhart PW, Smulders MJM

(2001) Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity*, **86**, 598–608.

Ward RD, Skibinsky DOF, Woodwark M (1992) Protein heterozygosity, protein structure and taxonomic differentiation. *Evolutionary Biology*, **26**, 3–131.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wright S (1951) The genetic structure of populations. *Annuals of Eugenics*, **15**, 323–354.

Wright S (1969) *Evolution and the Genetics of Populations*, Vol. 2. *The Theory of Gene Frequencies*. University of Chicago Press, Chicago.

The main focus of the authors' current research is in studies which aim to combine methods of molecular and statistical genetics to make inference about microevolutionary processes in populations of wild vertebrates.

Appendix I

Allele frequencies in eight microsatellite loci in six common frog populations used in this study

	Lund	Uppsala	Umeå	Ammarnäs	Kiruna	Kilpisjärvi
RRD590						
171	0.015	0	0	0	0	0
181	0.015	0	0	0	0	0
183	0.833	0.756	0.889	0.970	0.530	0.122
189	0	0.244	0	0.030	0.318	0.171
213	0.136	0	0.111	0	0.152	0.707
N	*33*	*41*	*36*	*33*	*33*	*41*
Rt2Ca2–22						
97	0.636	0.095	0.875	0.186	0.500	0.757
99	0.273	0.905	0.125	0.800	0.500	0.243
105	0	0	0	0.014	0	0
125	0.015	0	0	0	0	0
127	0.061	0	0	0	0	0
129	0.015	0	0	0	0	0
N	*33*	*42*	*36*	*35*	*36*	*37*
Rt2Ca25						
111	0	0	0	0	0.264	0.213
113	0.324	0.176	0	0.353	0.028	0
115	0.029	0	0	0	0	0
117	0	0	0	0.015	0	0
123	0.235	0.595	0.347	0	0.111	0
125	0.029	0	0.014	0	0	0
127	0	0	0.056	0.309	0.125	0
129	0	0	0	0.147	0	0
135	0.029	0.027	0	0.015	0	0.050
137	0.015	0	0	0.015	0	0
139	0.059	0	0	0	0.014	0.213
141	0.015	0	0	0	0.014	0.050
143	0.074	0	0.014	0	0	0.075
145	0	0	0	0	0	0.100
147	0.044	0	0.042	0	0	0
149	0.029	0	0.306	0	0.014	0.013
151	0.074	0	0.028	0	0.028	0.063
153	0	0.014	0.056	0.015	0.069	0.125
155	0.015	0.054	0.042	0.074	0.125	0.063
157	0.029	0.041	0.028	0	0.111	0.025
159	0	0.014	0	0	0	0.013
161	0	0	0.069	0.059	0	0
163	0	0.081	0	0	0.097	0
N	*34*	*37*	*36*	*34*	*36*	*40*
Rtempµ4						
146	0.063	0	0	0	0	0
148	0.141	0	0.083	0	0.206	0.131
150	0	0.016	0.222	0	0.250	0
152	0	0.031	0	0	0	0
154	0.313	0.219	0.014	0	0.015	0.012
155	0.047	0	0	0	0	0
156	0.047	0	0	0	0	0.048
158	0.016	0	0.111	0.339	0.206	0.524
160	0	0	0	0	0	0.155
162	0.063	0.063	0.417	0.355	0.324	0.083
164	0	0.031	0.111	0.129	0	0
166	0	0.047	0	0.016	0	0
168	0	0	0	0	0	0.048
174	0	0	0	0.161	0	0

© 2003 Blackwell Publishing Ltd, *Molecular Ecology*, 10.1046/j.1365-294X.2003.01865.x

Appendix I *Continued*

	Lund	Uppsala	Umeå	Ammarnäs	Kiruna	Kilpisjärvi
176	0.016	0	0	0	0	0
178	0.016	0.047	0.042	0	0	0
180	0.063	0	0	0	0	0
182	0.063	0.172	0	0	0	0
184	0.016	0.047	0	0	0	0
186	0	0.047	0	0	0	0
190	0.016	0.234	0	0	0	0
192	0.031	0.047	0	0	0	0
196	0.031	0	0	0	0	0
198	0.016	0	0	0	0	0
200	0.047	0	0	0	0	0
N	*32*	*32*	*36*	*31*	*34*	*42*
Rtempµ5						
228	0	0	0.028	0	0.029	0
232	0.333	0	0	0	0	0
242	0.030	0.345	0.417	0.771	0.414	0.049
244	0	0	0	0	0	0.012
246	0	0	0.014	0	0	0
264	0.121	0.155	0	0	0	0
268	0.061	0.103	0	0.021	0.200	0.402
270	0	0	0	0	0.029	0
272	0	0	0.111	0.208	0.314	0.402
276	0.091	0	0.181	0	0	0.134
278	0	0.017	0.222	0	0	0
280	0.364	0.379	0.028	0	0.014	0
N	*33*	*29*	*36*	*24*	*35*	*41*
Rtempµ7						
74	0.056	0	0	0	0	0.073
88	0.458	0.119	0.181	0.779	0	0
90	0.069	0.012	0	0	0	0
108	0.111	0.286	0	0	0	0
110	0.014	0	0	0	0	0
112	0.014	0.012	0	0	0	0.012
114	0.014	0.024	0.083	0	0.056	0.220
116	0.153	0.036	0.014	0.059	0.347	0.671
118	0.097	0.512	0.681	0.162	0.597	0.012
120	0.014	0	0	0	0	0.012
122	0	0	0.042	0	0	0
N	*36*	*42*	*36*	*34*	*36*	*41*
RtµH						
205	0.588	0.667	0.014	0.152	0	0
206	0.132	0	0.257	0	0	0
207	0.015	0	0	0	0	0
209	0.103	0	0.343	0.174	0.314	0.463
211	0	0	0.286	0.348	0.686	0.500
213	0.088	0.333	0.100	0.326	0	0.037
215	0.074	0	0	0	0	0
N	*34*	*36*	*35*	*23*	*35*	*41*
RtSB03						
295	0.032	0.348	0.222	0.034	0	0
297	0	0	0	0.069	0	0
307	0.048	0.106	0	0	0	0
311	0.129	0.045	0	0.466	0.083	0.278
313	0.081	0	0	0.034	0.097	0.028
315	0.016	0	0	0	0	0.014
317	0.339	0.015	0.208	0	0	0
318	0	0	0	0.017	0.042	0

Appendix I *Continued*

	Lund	Uppsala	Umeå	Ammarnäs	Kiruna	Kilpisjärvi
319	0.016	0.121	0.181	0.310	0	0.431
321	0.032	0	0	0	0.028	0.014
322	0	0	0.056	0	0.306	0.014
323	0.032	0.288	0	0	0.069	0.028
324	0.032	0.015	0.236	0.052	0.042	0.181
325	0.097	0	0	0	0.042	0
326	0.032	0	0.028	0	0.069	0
327	0.065	0.061	0.014	0.017	0.069	0
328	0	0	0.056	0	0.153	0
329	0.048	0	0	0	0	0
331	0	0	0	0	0	0.014
N	*31*	*33*	*36*	*29*	*36*	*36*