

Comprehensive Summaries of Uppsala Dissertations
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Local Adaptation, Countergradient
Variation and Ecological Genetics of
Life-history Traits in *Rana*
Temporaria

BY

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ACTA UNIVERSITATIS UPSALIENSIS
UPPSALA 2003

Dissertation for the Degree of Doctor of Philosophy in Population Biology presented at Uppsala University in 2003

ABSTRACT

Laugen, AT 2003. Local adaptation, countergradient variation and evolutionary genetics of amphibian populations. Acta Universitatis Upsaliensis, *Comprehensive Summaries of Uppsala Dissertation from the Faculty of Science and Technology* 818. 33 pp. Uppsala. ISBN 91-554-5558-1

The main aim of this work was to identify local adaptation processes in amphibian populations, thereby improving the general understanding of genetics and mechanisms behind the evolution and maintenance of biological diversity. Phenotypic and genetic variation in life-history traits was studied within and between populations common frog (*Rana temporaria*) populations along a 1600 km transect from southern Sweden to northern Finland.

Embryonic and larval development and growth was investigated both under field and laboratory conditions. The results suggest ample genetic diversity in larval life-history traits among Fennoscandian common frog populations. Larval developmental rate along the gradient has evolved a countergradient variation pattern of genotypes and phenotypes as indicated by the positive relationship between developmental rate and latitude under laboratory conditions and the lack of such a relationship in the field. The data suggest that this pattern has evolved because of time constraints due to decreasing length of growth season with latitude. Neither field-caught adults nor laboratory raised larvae displayed a linear latitudinal size cline as expected from the so called Bergmanns rule. Rather, size increased towards the mid-latitude populations and decreased thereafter, indicating that body size is a product of direct environmental induction or a trade-off with other life-history characters. Age and size at hatching showed no consistent latitudinal pattern, indicating that the embryonic stage is not as time constrained as the larval stage.

A large part of the variation in age and size at metamorphosis among populations was due to additive genetic effects. However, small, but significant maternal effects, mostly due to variation in egg size and non-additive genetic effects also contributed to among population variation. A comparison of divergence in presumably neutral molecular genetic markers (F_{ST}) and quantitative characters (Q_{ST}) revealed that although both estimates of divergence were relatively high, estimates of Q_{ST} was generally higher than those of F_{ST} , indicating that the genetic variation observed in larval traits is primarily a result of natural selection rather than genetic drift. Hence, our results reinforce the conclusion that intraspecific genetic heterogeneity in the young northern European ecosystems may be more widespread than previously anticipated

Key words: amphibians, Bergmanns rule, body size clines, countergradient variation, development rate, F_{ST} , local adaptation, temperature, Q_{ST} .

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ISSN 1104-232X

ISBN 91-554-5558-1

Printed in Sweden, Akademityck AB, Edsbruk

The thesis is based on the following articles, which are referred to by their Roman numerals in the text

- I** Laugen AT, Laurila A & Merilä J. Latitudinal and temperature-dependent variation in embryonic development and growth in *Rana temporaria* Oecologia, in press.
 - II** Laugen AT, Laurila A, Räsänen K & Merilä J. Latitudinal countergradient variation in the common frog (*Rana temporaria*) developmental rates – evidence for local adaptation. Revision submitted to Journal of Evolutionary Biology.
 - III** Laugen, AT, Laurila A, Jönsson JI Söderman F & Merilä, J. Does Bergmann rule? Latitudinal size variation in *Rana temporaria* revisited: Manuscript.
 - IV** Laugen AT, Laurila, A & Merilä, J. Maternal and genetic contributions to geographic variation in larval life history traits in common frogs (*Rana temporaria*). 2002. Biological Journal of the Linnean Society 76: 61-70
 - V** Palo JU, O'Hara RB, Laugen AT, Laurila A, Primmer CR & Merilä J. Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative data. Revision submitted to Molecular Ecology.
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INTRODUCTION.....	7
METHODS.....	9
Study species and populations	9
Field investigations	11
Rearing conditions of embryos and larvae	11
Response variables.....	12
Experimental design and statistical analyses	13
Molecular genetic analyses.....	14
RESULTS AND DISCUSSION	15
Countergradient variation in developmental rates (I, II)	15
Size variation: Does Bergmann rule? (I, III).....	17
Genetic or maternal effects ? (IV).....	20
Natural selection vs genetic drift (V).....	22
GENERAL CONCLUSIONS.....	25
ACKNOWLEDGEMENTS.....	26
REFERENCES.....	28

INTRODUCTION

"Then I saw three evil spirits that looked like frogs..."

Revelation 16:13

One of the major challenges in evolutionary biology has been to understand the causes and mechanisms of local adaptation. This is partly connected to the intrinsic interest of unravelling the past evolutionary history of extant taxa and populations, and partly to the wish for predicting the future evolutionary response of populations and ecosystems to a changing environment (Mousseau 2000). Spatial environmental heterogeneity is repeatedly established as a factor contributing to creation of genetic heterogeneity (Mayr 1963; Antonovics 1971; Hedrick 1976; 1986; Endler 1986; Linhart and Grant 1996; Reznick and Ghalambor 2001), and fluctuations in biotic and abiotic factors over time are believed to have had large impact on populations and species through mass extinctions and evolution of new varieties. Still, the actual selection regimes of different populations are seldom known, and the relative role of natural selection versus stochastic forces such as genetic drift as a driving force behind any observed differentiation has so far mainly been a matter of speculation. However, comparisons of divergence in neutral marker genes (as measured by F_{ST}) and genes coding quantitative traits (as measured by QST ; 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 and Crnokrak 2001; McKay and Latta 2002).

Climatic factors are potential sources of selection, especially in ectothermic species. For instance, temperature varies relatively systematically with latitude and elevation, creating ample opportunity for local adaptation along latitudinal clines, especially in ectothermic species. According to the temperature adaptation hypothesis (Levinton and Monahan 1983; Lonsdale and Levinton 1985), organisms are adapted to the temperature they most commonly experience in the natural environment. Alternatively, time constraints in the form of a short growth season imposed by low temperature, may select for fast growth or development, creating a countergradient variation pattern of genotypes in which genetic and environmental influences on a trait oppose each other along an environmental gradient (Levins 1969; Conover and Schultz 1995). Such a negative covariance between environmental and genetic influences may give a superficial impression of lack of genetic differentiation, and conceal even substantial genetic differences along the gradient. Countergradient variation in growth and developmental rates in responses to climatic variation has been demonstrated in a number of

ectotherms (Berven et al. 1979; Berven 1982a,b; Conover and Present 1990; Blanckenhorn and Fairbairn 1995; Parsons 1997; Jonassen et al. 2000). Temperature has also proven to shape of body size clines in ectotherms, but results differ regarding the direction of the correlation between size and temperature (Van Voorhies 1996, 1997; Partridge and Coyne 1997; Belk and Houston 2002) and therefore there has been considerable debate whether ectotherms follow the so called Bergmann's Rule and whether size clines are adaptive or just mere consequences of direct environmental induction.

Another important feature of geographic life history variation is the role of parental effects, defined as 'any parental influence on offspring phenotype that cannot be attributed solely to offspring genotype, to the direct action of the non-parental components of the offspring's environment, or to their combination' (Lacey 1998), as determinants of observed patterns. Parental effects, commonly occurring through environmentally mediated maternal effects, are often adaptive (reviewed by Mousseau and Fox 1998). Mothers may increase offspring fitness through mechanisms such as investment in resources to propagules (Bernardo 1996a) or choice of favourable oviposition sites (Bernardo 1996b). Environmental conditions experienced by the mother may affect the offspring's performance for several generations (Lacey 1998) and environmental variation may play a prominent role in the expression of maternal effects (Rossiter 1998). One important path of maternal effects influencing offspring phenotype in ectotherms is egg size: larger eggs produce larger and faster developing offspring with potentially far-reaching consequences for fitness (see Mousseau and Fox 1998 for reviews). In most ectotherms, egg size tends to increase with decreasing temperature or with increasing altitude and latitude (e.g. Berven 1982b; Azevedo et al. 1996; Yampolsky and Scheiner 1996). Consequently, unless egg size effects are accounted for, any genetic differentiation along a climatic gradient may become confounded with maternal effects acting through egg size. Despite this, the role of egg size in creating geographic gradients in offspring phenotypes has remained largely unexplored (Bernardo 1996a).

Amphibians possess several attributes that make them a useful model system for studies of spatial genetic differentiation in ecologically important traits. Firstly, amphibian species are generally poor dispersers. Mark-recapture and tracking studies have shown that many frog and toad species only rarely disperse further than five kilometres from the natal ponds (Reading et al. 1991; Sinsch 1997). However, these studies are based on relatively small sample sizes and the maximum migration distances may be larger. Furthermore, indirect evidence suggests limited dispersal in this group of animals: amphibian populations are genetically more structured than any other animal populations (Ward et al. 1992)

and results from molecular studies confirm to predictions from isolation-by-distance models (Storfer 1999). Secondly, amphibians are well-suited organisms for quantitative genetic studies required for establishing the genetic basis of among-population differentiation in phenotypic traits. The possibility of raising a large number of eggs and larvae (and in some species also juveniles and adults) under controlled laboratory conditions make common garden experiments and quantitative genetic analyses feasible. Finally, amphibian populations are declining on a global basis, a fact that has evoked world-wide attention (Barinaga 1990; Blaustein and Wake 1990; Borchelt 1990; Vitt et al. 1990; Wake 1991) and amphibians are now regarded as potentially good bioindicators of environmental health (Dunson et al. 1992; Fellers and Drost 1993; Blaustein et al. 1994). Hence, there is immediate concern about the adaptability and persistence of many amphibian populations in the face of habitat fragmentation and new selection pressures imposed by environmental changes.

The aims of this thesis were: First, to quantify geographic variation in growth and development in common frog, *Rana temporaria*, embryos (I), larvae (II, III,) and adults (III) and investigate the relative importance of genetic divergence and environmental factors in shaping phenotypic patterns among populations in embryos (I) and larvae (II, III) along a 1600 km latitudinal gradient across Fennoscandia. Second, to investigate the importance of genetic and maternal effects on phenotypic variation between populations (IV). Third, to investigate the role of natural selection and genetic drift in creating phenotypic variation among the latitudinal populations (V).

METHODS

“Nevertheless frogs and toads have few admirers even amongst professed zoologists”
Mivart, 1874

Study species and populations

Rana temporaria is the most widespread anuran in Europe (Gasc et al. 1997), and thereby subject to widely differing environmental conditions in different parts of its distribution range (Miaud et al. 1999; Miaud and Merilä 2001). Consequently, the opportunity for local differentiation among populations should be large. *Rana temporaria* breed further north and at higher altitudes than any other European anuran (Gasc et al. 1997). The larval period in the wild lasts about 35-100 days, depending on climatic conditions.

The populations studied along the latitudinal gradient are shown in Fig 1. The onset of breeding season between the southern- and northernmost localities differs by approximately 60 days, and there is a two-fold difference in the length

of growth season between these localities. Mean daily ambient temperatures over the past 26 years (1976-2002; data from the Swedish Meteorological and Hydrological Institute and the Finnish Meteorological Institute) during 30, 60 and 90 day periods following onset of egg laying in a given locality are shown in Fig. 2. Mean ambient temperatures during the first 30 days following the onset of breeding tend to increase with latitude, but by the time the first metamorphs appear in the majority of the ponds, temperature peak at mid-latitudes and is about equal at both ends of the latitudinal gradient (Fig. 2). When metamorphosis has passed its culmination (90 days after breeding onset), mean ambient temperatures over the developmental period are already much lower in north than in south (Fig. 2). Consequently, when viewed over the entire period of development, the northernmost frogs experience similar or lower mean ambient temperatures than the more southern frogs. All study ponds were small to medium sized without shading by canopy cover, supported breeding populations of 50-150 females.

Table 1. Descriptive information about the eight populations from which the adult data is collected.

Area	Latitude	Longitude	Papers
Lund	55°43'	13°26'	I, II, III, IV, V
Blekinge	56°18'	14°33'	III
Värmland	59°28'	13°31'	III
Uppsala	59°51'	17°14'	I, II, III, V
Umeå	63°52'	20°13'	I, II, III, IV, V
Ammarnäs	65°54'	16°18'	III, V
Kiruna	67°51'	21°02'	I, II, III, V
Kilpisjärvi	69°03'	20°47'	I, II, III, V



Figure 1. Map of all the populations used in the thesis

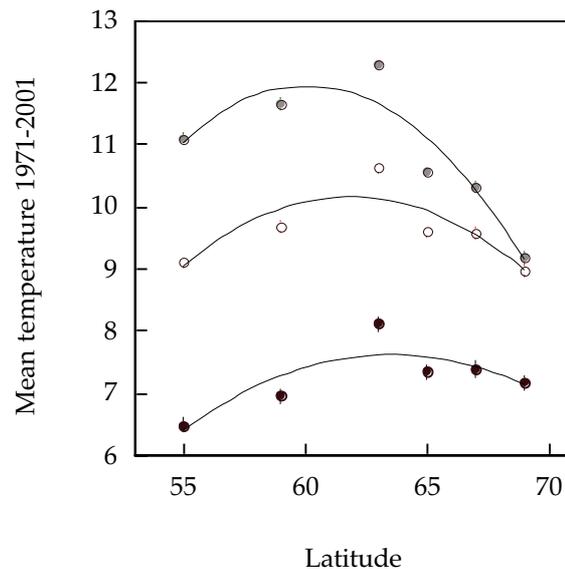


Figure 2. Mean \pm s.e. daily ambient temperatures over 30, 60 and 90 day periods following onset of spawning in a given population as a function of latitude (1976-2001). The start dates for spawning in each population are: Lund 1st April; Uppsala 15th April; Umeå 5th May; Ammarnäs 15th May; Kiruna 25th May and Kilpisjärvi 1st June. Filled dots represent 30 days, open dots represent 60 days and grey dots represent 90 days following spawning

Field investigations

Adults were captured at breeding sites early in the breeding season and were transported to Uppsala where they were used as parental generation in breeding experiments. Age of individual amphibians was determined by counting the number of yearly bone growth layer rings. In each year, a band of bone tissue is formed bordered by a dark line of arrested growth (Castanet et al. 1977). Sections of the second phalange of the right hind leg were prepared with standard skeletochronological methods (Hemelaar 1981;1985). Sections were photographed with a digital camera fitted to a stereomicroscope, and the number of rings was determined by the use of a light microscope.

Information on larval development and growth from natural populations (II, III) was obtained by dip-netting 17 ponds from three geographical areas once a week until 20-30 tadpoles were sampled each week. The tadpoles were killed using MS-222 and stored in 70 % ethanol.

Rearing conditions of embryos and larvae

In the laboratory, embryos and tadpoles were obtained from artificial fertilisations to obtain homogenous material for estimation of genetic

components of variation within and between populations. Fertilisations were carried out following Berger et al. (1994) with some modifications. Sperm suspensions were prepared in Petri dishes with 3 ml of 10% amphibian Ringer solution (Rugh, 1962) and then moved to larger dishes, one per male. Eggs were stripped into the sperm solutions. After five minutes, more Ringer solution was added to cover the eggs and, after 20 minutes, the solution was replaced with reconstituted soft water (RSW; APHA 1985). One hour after fertilisation, eggs were gently detached from the dish and divided into several one-litre bowls where they were transferred to experimental temperatures (I), kept until hatching in 14, 18 and 22 °C (II, III, V) or in 14 °C (IV). Water in each bowl was changed every third day.

The embryonic stage, from fertilisation to hatching (complete gill absorption; Gosner stage 25; Gosner 1960; I) and the larval stage, from hatching to metamorphosis (emergence of first forelimb; Gosner stage 42; Gosner 1960; II, III, IV, V) were investigated. Embryos (I) were reared at seven different temperature treatments (9, 12, 15, 18, 21, 24, 27 °C) arranged in water bath systems (one per temperature) in which heater or cooling units kept the temperature at the desired level. Embryos were raised in 0.9 litre vials containing reconstituted soft water (RSW). The density was about 50 eggs per vial. Larvae were raised in either one (IV) or three (II, III, V) different temperatures that were kept constant in climate rooms. The larvae were raised singly in 0.9 litre vials. They were given a finely ground 1:3 mixture of fish flakes and rodent pellets every seventh (II, III, V) or every fourth (IV) day. The amount of food given to each tadpole was 15 (restricted) and 45 (*ad libitum*) mg for the first feeding event, 30 and 90 mg for the second, and 60 and 180 mg from the third feeding event thereafter until metamorphosis (II, III, V) or 30 and 90 mg from the second feeding and thereafter until metamorphosis (IV). Tadpoles were raised in RSW that was changed every seventh (II, III, V) or every fourth (IV) day in conjunction with feeding.

Response variables

In paper I, embryonic survival, age and total length at hatching and embryonic growth rate were used as response variables. Age at hatching was defined as the number of days elapsed between fertilization and Gosner stage 25 (Gosner 1960). Survival was the proportion of successfully hatched embryos to the number of successfully fertilised eggs. Size at hatching was determined by the use of a stereo microscope fitted with an ocular micrometer. From each field-caught larva, the developmental stage according to Gosner (1960) was determined. The developmental rate in the field (II) was defined as the number days elapsed

between the day when the first hatchlings reached Gosner stage 25 and the day when the first larvae reached Gosner stage 42 for each pond. Since this method relies heavily on observations of single individuals, the mean developmental stage ca 40-50 days after the larvae had reached the Gosner stage 25 was used as an additional measure of developmental rate in the wild. Size of field-caught metamorphs and adults (**III**) was measured as snout-vent length using digital callipers to the closest 0.01 cm.

In the laboratory experiments in paper **II**, **III**, **IV** and **V**, age and size at metamorphosis was used as response variables. Age at metamorphosis was defined as the time elapsed between Gosner stage 25 and 42, and size was measured as body weight to the nearest 0.0001 gram. In paper **IV** growth rate was defined as size/age, whereas in paper **V** growth rate was modelled by fitting age at metamorphosis as a covariate in the analysis of size at metamorphosis and analysed by using the residuals. Mean female specific egg size was used as a covariate to investigate the effects on embryonic (**I**) and larval (**II**, **IV**) traits. Egg size was measured in two ways. In paper **II**, a sample of newly fertilised eggs was stored in 4 % formaldehyde and was later measured with a stereo microscope fitted with an ocular micrometer. In papers **I** and **IV**, egg size were measured from photographic images by using NIH IMAGE software (<http://rsb.info.nih.gov/nih-image>).

Experimental design and statistical analyses

In paper **I**, a maternal half-sib design (North Carolina I, Lynch and Walsh 1998) was used to investigate the effects of dam and sire on embryonic traits. Five males were each mated to two females. In paper **II** and **III**, eight females were each mated to two males (=16 males used) except for the Umeå and Ammarnäs populations, and the mean value of the two maternal half-sib families were used in the analyses. In the Umeå population, 16 females and 32 males were used, whereas in the Ammarnäs population, eight freshly laid spawn clumps were collected and brought to the laboratory in Uppsala. In paper **IV**, reciprocal crosses of two latitudinally separated populations were used to investigate the relative importance of additive and non-additive genetic variation and maternal effects on population differences in larval growth rate and age and size at metamorphosis. Full-sibships were created with four different parentages: LL: southern female - southern male, LU: southern female - northern male, UL: northern female - southern male and UU: northern female - northern male. The interpretation of the different effects was as follows. If the population differences in response variables were mainly due to population specific maternal effects, tadpoles from the same maternal parentages (cf. LL and LU or UU and UL) were

expected not to differ in their mean values. If, however, the differences were largely due to additive genetic effects, the offspring from reciprocal crosses (LU and UL) were expected to be intermediate in relation to the within population crosses (LL and UU), and the means of the reciprocal crosses to be similar. Deviations from the intermediate pattern would be indicative of non-additive genetic influences. The randomised block experiments in paper I, II, III and IV were analysed with factorial general linear models (PROC GLM) and general linear mixed models (GLMM) as implemented in the SAS statistical package (version 6.12, SAS Inst., Inc.).

In paper V, an incomplete North Carolina II breeding design (Lynch & Walsh 1998) was used to investigate genetic architecture in larval traits enabling estimation of within and among population components of genetic variation in larval life history traits. In all but the Umeå and Ammarnäs populations, 32 full-sib/half-sib families were created where eggs from each of eight females were fertilised by sperm from four of the 16 males. The Umeå tadpoles stem from 32 maternal half-sib families (16 females and 32 males used). For the Ammarnäs population, eight freshly laid spawn clumps were collected from the wild. To estimate the variance components, an animal model was fitted with a Bayesian approach (Gelman et al. 1995) using a Gibbs sampler in the WinBugs package (Spiegelhalter et al. 1999). Divergence among populations in quantitative characters were assessed by estimation of Q_{ST} measured as:

$$Q_{st} = \frac{V_{GB}}{2V_{GW} + V_{GB}}$$

(Merilä and Crnokrak 2001) 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 in question.

Molecular genetic analyses (V)

To obtain estimates of population differentiation (F_{ST}) in neutral markers, allelic variation was assessed for 36 to 42 randomly sampled adult individuals from six populations in eight microsatellite loci. DNA was extracted using standard SDS – proteinase K digestion treatment followed by isopropanol precipitation (e.g. Bruford et al. 1992), and after PCR amplifications, the PCR products for each individual were resolved by denaturing 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 Mousadaik and 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} ; statistical significance of which was tested using the randomisation procedure implemented in FSTAT v.2.9.3 (Goudet 2001). The amount of differentiation of allele frequencies between each pair of populations was quantified using Weir and Cockerham's (1984) standardized F_{ST} .

RESULTS AND DISCUSSION

"What is a frog? At first, almost all persons will think, on meeting with this question, that they can answer it readily and easily. Second thoughts, however, will show to most that such is by no means the case"
Mivart, 1874

Countergradient variation in developmental rates (I, II)

At higher latitudes selection to complete development before the onset of winter should be strong, and increased uptake rate and digestion efficiency has been demonstrated in northern fish populations (Present and Conover 1992; Jonassen et al. 2000). Accordingly, when raised in a common environment, northern populations are expected to develop faster (Conover and Schultz 1995).

Embryos from the different populations differed significantly in developmental rate (Fig. 3; I), but there was no consistent linear trend along the gradient that would indicate a countergradient variation pattern in embryonic development. Temperature had a strong effect on development time, which decreased by roughly 70 % from 9 to 24 °C (Fig. 3), and the different populations reacted differently on the temperature treatments. The lack of latitudinally ordered variation in embryonic traits contrasts to the studies on larval life-history variation in this and many other ectothermic species. Previous evidence suggests that embryonic development rate in ectotherms may increase towards higher latitudes (Groeters and Shaw 1992; Dingle and Mousseau 1994; DiMichele and Westerman 1997). Similarly, studies on *R. temporaria* have shown that embryos from low altitude populations have slower developmental rates than those from higher altitudes (Angelier and Angelier 1968; Martin and Miaud 1999). However, like the present study, Pakkala et al. (2002) did not find any clear pattern in embryonic development time across a Scandinavian latitudinal cline. It seems possible that the time constraint imposed on embryonic development rate is not as severe as in larval development (see below), possibly because larvae may be able to later compensate for delays in hatching (Räsänen et al. 2002).

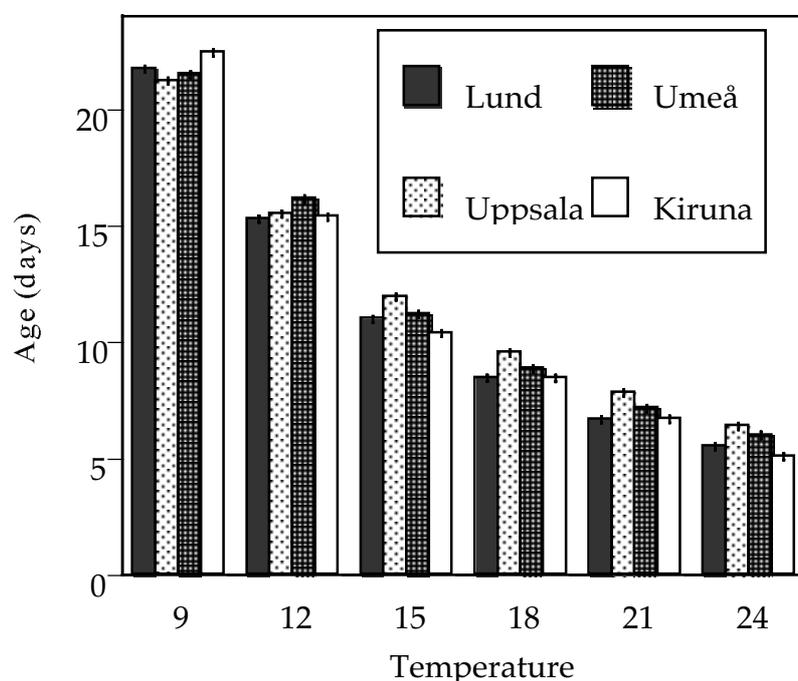


Figure 3. Mean embryonic development time in four *R. temporaria* populations in six temperature treatments. The values represent least square means \pm SE.

In the laboratory study of larval development (II), there was a strong negative relationship between latitude and age at metamorphosis in all temperatures and in both food treatments (Fig. 4). Larval period was roughly ten days shorter in the northernmost population as compared to the southernmost population (Fig. 4), but the exact magnitude of this difference depended slightly but significantly on temperature. In general, increasing temperature had a large accelerating effect on developmental rate in both food levels and in all populations (Fig. 4): larval period was 30-40 days longer at 14 °C than at 22 °C. Although developing faster in the laboratory, the northern frogs did not realise their genetic capacity for fast development in the field, where mean estimated age at metamorphosis did not differ significantly among the three latitudinal areas (II). Variation among ponds was large, and the range of values recorded for ponds from the southernmost latitudinal area fell into the range values recorded in the northernmost area. This is to be expected if the local environmental effects, such as variations in temperature and food availability, override the genotypic effects. Hence,

whatever the proximate cause for the large variation in developmental rates in the field, it effectively concealed the genotypic differentiation, resulting in a countergradient variation pattern (Conover and Schultz 1995).

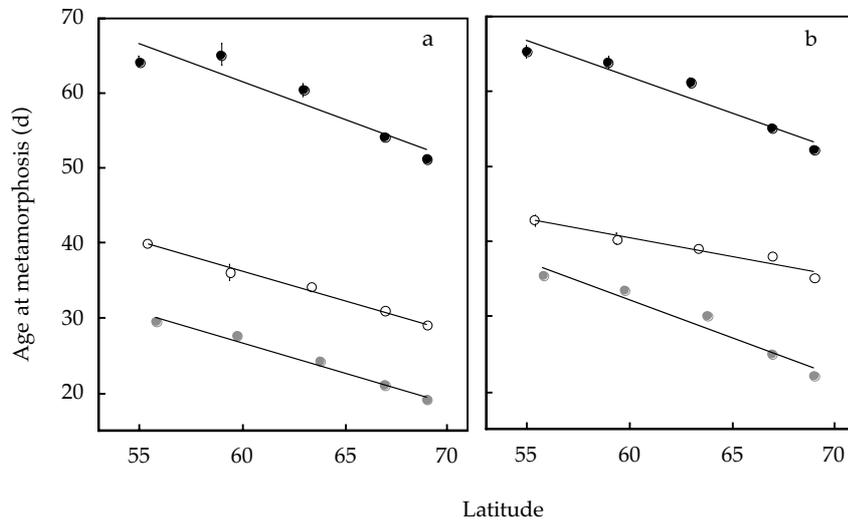


Figure 4. Effects of population of origin, temperature and food treatments on age at metamorphosis. Mean age (\pm S.E.) as a function of latitude of origin under (a) *ad libitum* and (b) restricted food levels. Filled dots represent means at 14 °C, open dots means at 18 °C and grey dots means at 22 °C.

Size variation: Does Bergmann rule? (I, III)

Bergmann's rule states that animal body size increases with latitude or decreasing ambient temperatures. In paper I, I found no evidence for a clear latitudinal pattern in hatchling size, but populations reacted differently to the different temperature treatments. For instance, hatchling size in the northern populations was clearly largest at the two highest temperatures. In their natural habitats, these populations experience higher mean temperatures under embryonic development, and my results suggest that they have capacity to exploit high temperatures to increase hatchling size in concordance with the temperature-adaptation hypothesis. Ståhlberg et al. (2001) found that early larval growth rates in *R. temporaria* were higher in a southern population at low temperature, whereas northern larvae grew better at higher temperatures lending support for temperature adaptation hypothesis. Hatchling size was smaller at low temperature, which is the opposite of the pattern commonly found in ectotherms (Atkinson and Sibly 1997, but see Mousseau 1997). This may be due to higher

cumulative energetic costs of development at low temperature. Energetic costs of embryonic development (when no external feeding occurs) are influenced by metabolic rate during development and the duration of the development. Thus, low temperatures may have lowered developmental rates to the point where increased energy expenditure affects body size negatively.

Along the latitudinal gradient across Scandinavia, body size of both adult *R. temporaria* caught from the wild (Fig. 5, III) and metamorphs raised in the laboratory (Fig. 6, III) peaked at the mid-latitudes and decreased towards the lowest and highest latitudes. Hence, while I found support for Bergmann's rule along the southern half of the gradient the pattern along the northern part was the converse to Bergmann's rule (Fig. 5). A possible explanation for this pattern in the larval stage is that the tadpoles from the northern populations trade-off larger metamorphic size against shorter larval period, i.e. the extremely short growth season in the northernmost populations may have favoured short larval period over larger body size. In paper II, I showed that larvae from northern populations of *R. temporaria* metamorphose faster than those from southern populations when raised in common environment. *R. temporaria* does not overwinter at the larval stage in Scandinavia (Miaud et al. 1999), hence, I suggest that the constraint on developmental time posed by short activity period can override the beneficial effects of larger metamorphic size, leading to reduced size at metamorphosis in the north. However, if large metamorphic size is beneficial in the absence of time constraints why do the southern tadpoles metamorphose at smaller size? Size at metamorphosis is predicted to reflect the balance between growth opportunities and survival between the aquatic and terrestrial habitats (Werner 1986; Rowe and Ludwig 1991). While the definite answer has to wait for further experiments, I suggest that the answer may lie in the relatively low growth rates in the south (Laurila et al. 2001) and, possibly, more favourable growth conditions in terrestrial habitat in the southern latitudes favouring metamorphosis at smaller size.

Many adaptive explanations for the occurrence of Bergmann's size clines have been put forward (see Ashton et al. 2000; Ashton 2002 for recent reviews). Ashton (2002) suggested that large body size in adult amphibians is associated with availability of water (precipitation and humidity). Therefore, larger individuals are favoured in the driest environments due to their greater tolerance to desiccation. However, variation in yearly precipitation is not very large along the present gradient (500-700mm; Alexandersson et al. 1991) and highest precipitation occurs in areas where the frogs are largest suggesting that variation in precipitation is not an important factor determining adult body size distribution in this case.

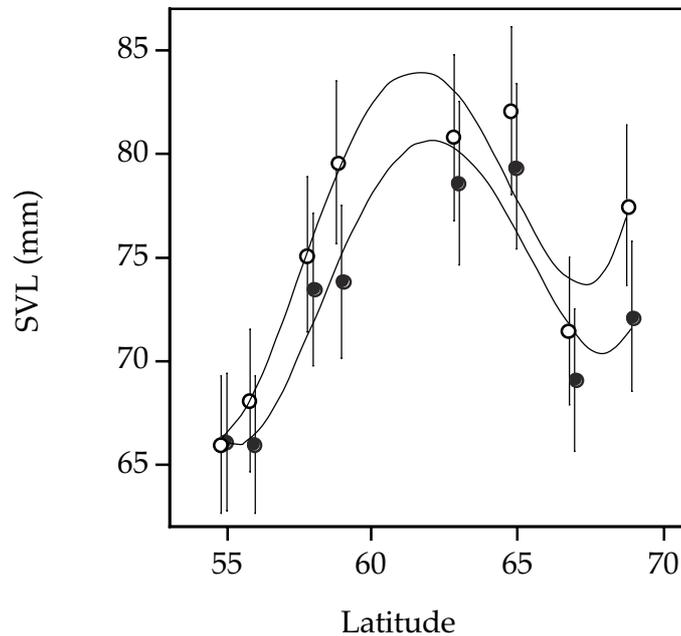


Figure 5. Mean \pm s.e. adult body size (snout-vent length) from eight populations of *Rana temporaria*. Filled dots represent males and empty dots represent females.

I found that low temperature increases metamorphic size in *R. temporaria* (III). These results add to the list of ectotherms showing largest size at low temperatures (Atkinson 1994; Ashton 2002). The mechanism creating this pattern has been a subject of much debate and is one of the major puzzles in the current theory of life history evolution (Atkinson and Sibly 1997), explanations ranging from temperature-dependent mortality (Yampolsky and Scheiner 1996) and physiological mechanisms of growth (Atkinson 1994; Perrin 1995) to the interplay between enzyme kinetics and biological rates (van der Have and de Jong 1996). As body size of metamorphs increases at low temperatures, the increased body size in adults towards higher altitudes and latitudes found in some studies of *R. temporaria* (Elmberg 1991; Miaud et al. 1999) and other amphibians (Ashton 2002) could also be, at least partly, explained by direct environmental induction. Berven et al. (1979) and Berven (1982a), two of the very few field studies of amphibian life-histories, found that tadpoles from mountain populations were larger than lowland tadpoles in natural ponds, but that the differences strongly diminished or even reversed when the tadpoles were raised in common garden experiments. Hence, the larger body size in mountain ponds was mostly a plastic

response to low temperature. The role of environmental induction in creating adult body size clines in amphibians remains open for further investigations.

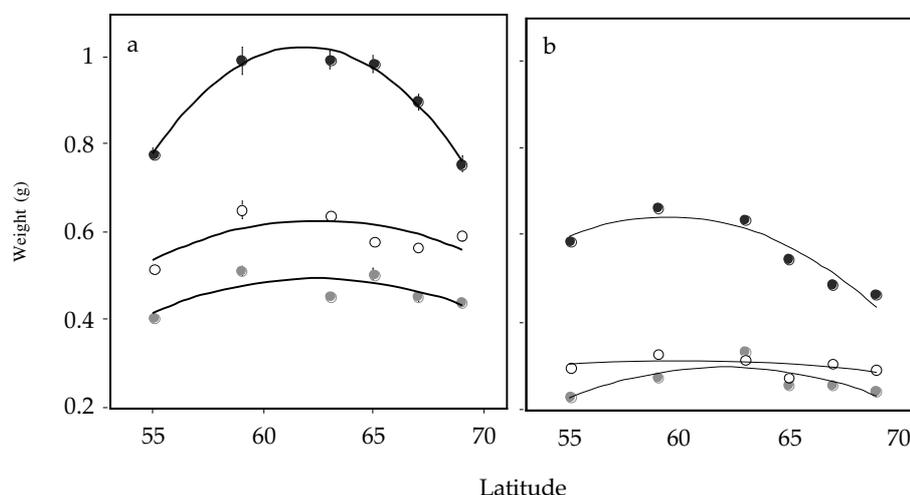


Figure 6. Effect of population, temperature and food level treatments on size at metamorphosis. Mean size \pm s.e. as a function of latitude of origin under a) *ad libitum* and b) restricted food levels. Filled dots represent means at 14 °C, open dots means at 18 °C and grey dots means at 22 °C.

Genetic or maternal effects ? (IV)

The reciprocal crosses suggested that both additive and non-additive genetic as well as maternal effects are important in determining variation among populations in larval life-history traits in *R. temporaria*. Age at metamorphosis was significantly influenced by paternal origin (Fig 7a), indicating a genetic basis for population differentiation in this trait. The fact that only one of the reciprocal crosses was intermediate relative to the intrapopulation crosses suggests that these genetic influences were not completely additive (Fig 7a). However, it is not entirely clear from my data whether these effects owe to non-additive gene interactions, or to interactions between genetic influences and maternal effects (Rossiter 1998). It would be tempting to conclude that the lack of female origin and egg size effects (Fig. 7a,b) suggest a minor role for maternal effects as determinants of divergence in timing of metamorphosis between these two populations. However, this conclusion would be premature in the face of the complexity revealed by the reciprocal crosses. Laurila et al. (2002) found generally weak maternal effects on larval developmental rate in one low and one high latitude *R. temporaria* population from the same gradient.

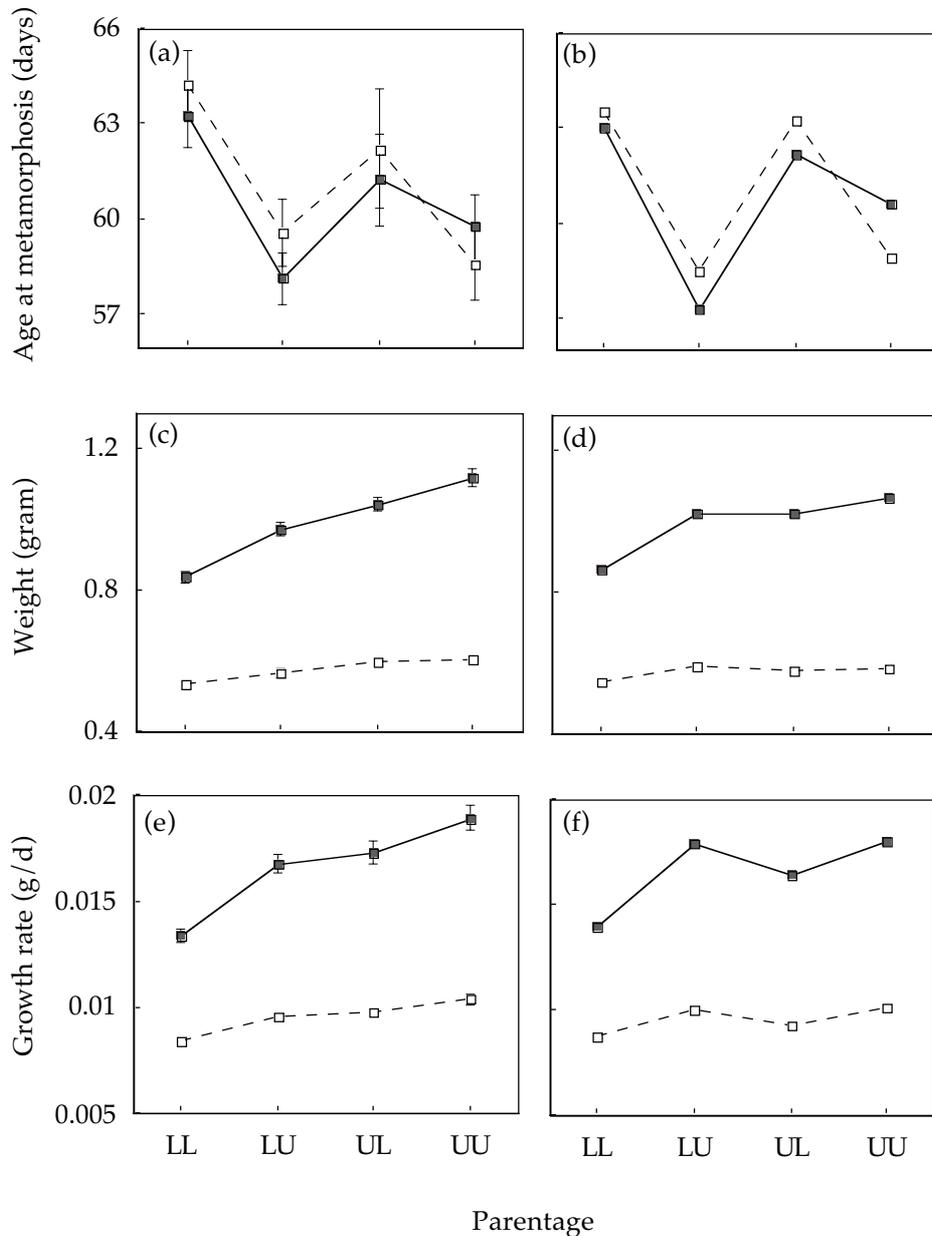


Figure 7. Mean (\pm SE) trait values (a,c,e) and least square means after accounting for egg size (b,d,f) from different crosses within and between different populations of *R. temporaria*. Top: age at metamorphosis, middle: size at metamorphosis and bottom: growth rate until metamorphosis. Solid lines and filled symbols refer to *ad libitum* food level, dotted lines and open symbols to restricted food level. LL = Lund female \times Lund male, LU = Lund female \times Umeå male, UL = Umeå female \times Lund male, UU = Umeå female \times Umeå male

The results for growth rate and size at metamorphosis were more readily interpretable. Evidence for an additive genetic basis of population differentiation in these traits was clear both in terms of intermediate values of reciprocal crosses and significant effects of both male and female origin on these traits (Fig 7c,d,e,f). However, in both traits, and in the case of size at metamorphosis in particular, the means of the two reciprocal crosses tended to converge towards the size of the maternal origin population. This, together with the fact that both traits were strongly influenced by egg size effects, suggests that part of differentiation in these traits is attributable to maternal effects. Interestingly, when egg size was introduced as a covariate, the differences in metamorphic size between parentages were larger under *ad libitum* food level than under restricted food level, bringing about a significant female origin \times food level interaction. Hence, the maternal effect contribution to size at metamorphosis seemed to be partly dependent on environment, but this became apparent only after the variation due to egg size was accounted for. Thus, our results support the contention that the expression of maternal effects may be highly context dependent (Berven and Chadra 1988; Semlitsch and Gibbons 1990; Kaplan 1992; Einum and Fleming 1999). In a similar way, the interactions between male and female origin effects became more pronounced (and significant) only after egg size effects were controlled for. These interaction effects could be due to non-additive gene action (but see above), but the fact that they were not detectable before the egg size effects were accounted for, suggests that they were weak relative to other sources of variation (additive and maternal effects) in these traits.

Natural selection vs genetic drift (V)

Among population differentiation at the marker loci was substantial, the overall F_{ST} being 0.235 (95% HPDI: 0.182 - 0.300). Pairwise estimates varied from 0.130 to 0.363 (Table 2), and the degree of differentiation tended to increase with increasing geographic distance between the populations as revealed by a positive correlation between the pairwise F_{ST} values and geographic distances ($r = 0.20$, 95% HPDI: 0.132- 0.281). 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. 8).

The degree of quantitative genetic differentiation in age at metamorphosis between different populations was positively correlated with geographic distance separating the populations (Fig. 9a). For the other two traits, there was no consistent relationship between quantitative genetic differentiation and

geographic distance (Fig. 9b,c). The degree of among population differentiation in quantitative traits was not consistently predictable from the degree of

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 (0.127 - 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)

differentiation in molecular markers (Fig. 9d-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. 9d), whereas for metamorphic size one significantly positive and two significantly negative relationships were observed (Fig. 9e). In the case of growth rate, only one of the correlations (positive) was significant (Fig. 9f). These findings clearly demonstrate that genotype-environment interactions can mask or enhance the correspondence between quantitative and molecular genetic measures of differentiation.

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 the 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 is also born out of the majority similar comparisons in other taxa (Merilä and Crnokrak 2001; bur

see: Lee and Frost 2002; McKay and Latta 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,

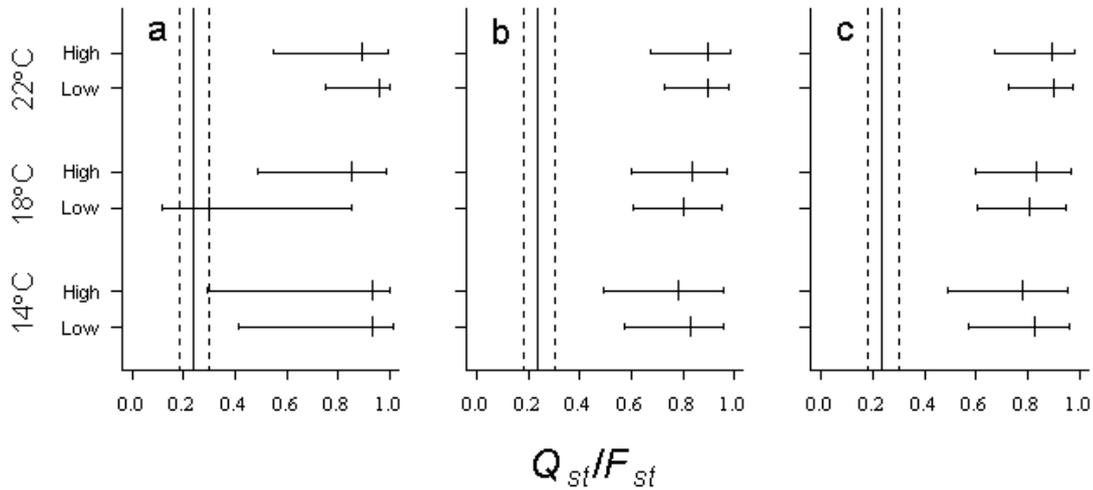


Figure 8. Comparison of overall Q_{ST} and F_{ST} estimates for (a) age, (b) body size, and (c) growth rate (i.e. body size corrected for age) under different treatment conditions. The vertical line depicts F_{ST} (± 95% C.I. – vertical dotted lines) and vertical bars Q_{ST} (± 95% HPDs – horizontal lines). ‘low’ and ‘high’ refer to low and high food level treatments.

unlike previous studies (but see: Koskinen et al. 2002)), I estimated Q_{ST} from a half-sib design, and as a consequence, Q_{ST} was not underestimated as will be the case for full-sib designs when maternal or dominance effects are present (Merilä & Crnokrak 2001). Secondly, in the case of the age at metamorphosis, which was the only trait showing a clear pattern of latitudinally ordered differentiation, the correlation between pairwise Q_{ST} estimates and geographic distances separating populations exceeded that between F_{ST} estimates and geographic 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 north is favoured due to shortening season length towards the north (II). Note that if the latitudinal cline in metamorphic age would be mainly driven by neutral divergence under an isolation by distance scenario, I would have expected concordant patterns of isolation by distance for both F_{ST} and Q_{ST} . Furthermore, I 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, my results suggest that the Q_{ST} estimates may be sensitive to genotype-environment interactions.

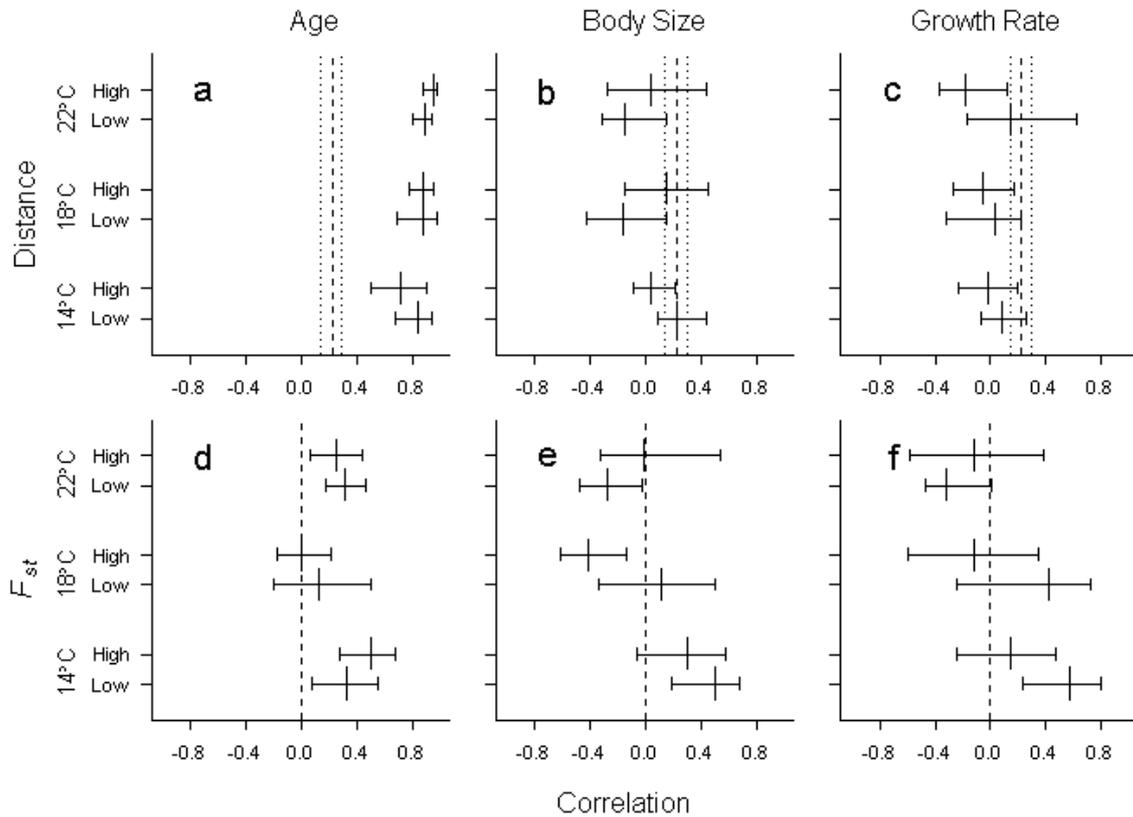


Figure 9. Posterior modes (\pm 95% HPDs) for correlations of pairwise comparisons of (a-c) $Q_{ST}/(1-Q_{ST})$ and geographic distance, (d-f) $Q_{ST}/(1-Q_{ST})$ and $F_{ST}/(1-F_{ST})$. In (a-c), the dashed line is mode of correlation between geographic distance and $F_{ST}/(1-F_{ST})$; dotted lines: 95% HPDs. In (d-f) the dashed line depicts a zero correlation.

GENERAL CONCLUSIONS

“It would take long indeed to tell the sufferings of much-enduring frogs in the cause of science!”

Mivart, 1874

My results demonstrate that the northern larval common frogs may have genetic capacity for faster development than the southern frogs, and this differentiation can be understood in terms of adaptation to seasonal time constraints that increase with latitude. The data on developmental rates from the wild show that the genetically faster development of the northern tadpoles is not realised under field conditions where environmental effects seem to override the genetic effects

rendering the observed divergence cryptic as predicted by the countergradient variation hypothesis. The lack of a countergradient variation pattern in embryos suggests that embryos are not under a heavy selection pressure to hatch early, possibly because delayed hatching can be caught up during the larval stage. I did not find any evidence for a genetically based body size cline among Scandinavian *R. temporaria* populations. This suggests that Bergmann's rule does not apply to this species, but that the growth of northern frogs is strongly constrained by low temperatures or short annual growth season.

Further, my results suggest that two geographically distinct *R. temporaria* populations have diverged genetically in larval life history traits. This genetic differentiation seems to be largely driven by additive action of genes, although one or more dominant alleles segregating in the northern population may be contributing to divergence in growth rate and size at metamorphosis. Also maternal effects, acting through egg size in particular, seem to contribute to the population divergence. However, caution is needed when extrapolating the results to other clines or species, as the relative importance of different causal components of variance in phenotypic traits may differ among different populations and clines of the same species.

In addition, I demonstrate a high degree of differentiation among relatively young common frog populations in both 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 north as compared to south. Hence, our results reinforce the conclusion that intraspecific genetic heterogeneity in the young northern European ecosystems may be more widespread than previously anticipated (Pamilo and Savolainen 1999).

ACKNOWLEDGEMENTS

"If you refuse to let them go, I will plague your whole country with frogs."

Exodus 8: 1

First of all I would like to thank my two supervisors (in order of appearance) Juha Merilä and Anssi Laurila for your support and for providing all the resources needed for the production of a thesis. You both have a sense of humour that has kept me up through tough times. Juha, I thank you for coming up with such a good project and letting me be part of it. You have always been supportive, and the effort you have put into this has been enormous. Anssi, thanks for your support and cooperation that has been invaluable (the Oecologia stunt was fab), for never losing your temper and for

giving me a systematic and very useful introduction to Finnish swearing and linguistic peculiarities like cows in ditches. You are more than a shoulder to cry on.

Special thanks also goes to all the members of “grodgruppen”; Katja for being such a patient co-worker and for being nearly as bad an inline skater as I am, Fredrik S. for helping with labwork, fixing Mac problems by just entering my office and for just being you; Bea for encouraging me to join a pysseljunta once in a while; Maarit for the office-sharing the first year, for mutual help in the lab and for many good laughs; Markus for your smiles that can melt rocks, Susanna for being cheerful, giving us a sofa and asking for help to move to Sthlm and thereby giving me an excuse to hit the road again in my 1972 Mercedes, Pierre-André for your lovely accent, Heidi for being so interested in football, all the lab assistants Constanze, Geir, Hanna, Satu, Karoliina, Kattis, Eevi, Mervi, Maaria and Peter, and especially Niclas for working nine to five and for making the labwork kind of fun. Thanks to Loeske Kruuk for coming up with the best piece of advice I got during the last two weeks of work with this thesis: “The only good thesis is a finished thesis” (ergo this IS a good thesis...). Thanks to Bea, Robert, Marcus, Jacob and Katja for comments on previous versions.

All of the people at the department have contributed to a friendly and productive environment. Special thanks goes to my professor JacobH who has been there when I needed money for (at least what I thought) important stuff. Thank you for last minute pep talks, for being so supportive during the last weeks of this project and of course for your, when necessary, p. i. outspokenness. Thanks to Marianne for always being able to get the hold of JanE when I needed his signature, to Nisse for helping me preparing the laboratory and enduring stressed frog-workers the last five years, to JohanW for being noisy elsewhere during the last four weeks of the production of this thesis, and to Robert and Jobs Karl for not being noisy in his absence. Thanks to all at the department for always being such fun at parties (especially after a certain amount of alcohol intake).

Maria inspired me to go to Uppsala in the first place and has always been there for me when I needed to share a bottle of wine or whisky. Thank you for being the best person to cheer me up (usually by leading me into some kind of shopping temptation). Stein Are has been part of my academic as well as my off-duty life for quite a few years now, and together with Maria you have added some continuity to my life; but I guess you’ll never be able to teach me to start preparing a talk before the very last minute. Thanks also to the whisky ladies for many good tasting events, to KatherineT, KarinL, AnnaK, SofiaB, FredrikW, ElinL, MarcusF, EmilN and all other skiers, snowboarders, party people and spexare for many great moments both on, off, after and away from pist, Nina, Lena and Kristin for being such good friends since I were seven years old, for always making holidays back home cheerful and for unforgettable moments in Osl, Sthlm & Cph the last three years (where do we go next?). My parents and my brother deserve thanks for always being eager to help with moving, money, car and giving me all the backup anyone could ever need. Lastly, I thank Geir for being such a good friend

and for being so patient and supportive during the last months of this ego-trip. Now it's your turn...

My work was financially supported by The Swedish Natural Science Research Council (to J. Merilä), The Royal Swedish Academy of Sciences, Helge Ax:son Johnsons Foundation and Uppsala University (The Zoological Foundation, Sederholms Nordiska Resestipendium, Gertrud Thelins resestipendium & Bjurzons resestipendium)

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