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ORIGINAL ARTICLE

Genetic variability predicts common frog (*Rana temporaria*) size at metamorphosis in the wild

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We investigated associations between genetic variability and two fitness-related traits – size and age at metamorphosis – in two subartic populations of the common frog, *Rana temporaria*. We found that metamorphic size was positively correlated with individual heterozygosity (as estimated using eight microsatellite loci) and that maternal heterozygosity also explained a significant amount of variation in this trait. In contrast, age at metamorphosis was only explained by environmental factors. Since size at metamorphosis is positively correlated with fitness in amphibians, these results suggest that genetic variability may be an important component of individual fitness in common frogs. The environmental variation underlying timing of metamorphosis may indicate that strong selection pressure on this trait in the Nordic environment is likely to override genetic effects.
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Introduction

Fitness and/or lifetime reproductive success, defined as the total number of offspring produced which reach maturity is extremely difficult to measure especially in taxa with external fertilization. In fact, two components of total reproductive success – reproductive lifespan and offspring survival – are practically impossible to measure in some cases because of difficulties in tracking individuals throughout their lifetime. As a result, indicators of fitness, which are easier to measure, but less accurate, are often used. For instance, reproductive output, that is, the number or size of offspring produced per mating event, is commonly used as a proxy for total reproductive success (Gibbons and McCarthy, 1986; Ryser, 1989), and several studies have investigated the variation in these traits to assess variation in individual fitness (Roff, 1992).

The relationship between fitness and genetic variability has often been investigated through associations between marker-based measures of genetic variability and fitness-related traits (e.g., Allendorf and Leary, 1986; Mitton, 1997; David, 1998; Lesbarrères *et al*., 2005). Rate of development or growth and survival have frequently been targeted in research, while investigation of reproductive success is more rare (but see McAlpine, 1993; Slate *et al*., 2000). In general, life history traits should exhibit high levels of dominance variance and should therefore be more strongly affected by inbreeding depression than more weakly selected morphometric traits (Roff, 1997; Merilä and Sheldon, 1999). Thus, assuming that the relationship between fitness and genetic variability results from effects of homozygosity at genome-wide distributed loci (i.e., general effect hypothesis; Hansson and Westerberg, 2002; Lesbarrères *et al*., 2005), there is potential for a link between genetic variability and reproductive success. Recent work (Balloux *et al*., 2004; Slate *et al*., 2004) has suggested problems in detection of genetic variability–fitness correlations (GFCs) at the individual level as well as difficulties in distinguishing between the underlying genetic causes (Tsitrone *et al*., 2001; Hansson and Westerberg, 2002). One problem is that when estimated from only a handful of markers, the correlation between genome-wide heterozygosity and its estimate obtained from these markers is very close to zero (Balloux *et al*., 2004). However, there are indeed some convincing case studies, which have been able to demonstrate GFCs using life history traits (Rowe *et al*., 1999; Markert *et al*., 2004; Lesbarrères *et al*., 2005; Da Silva *et al*., 2006).

In amphibians, while stochasticity is usually observed between the number of females present, the number of eggs deposited and the number of metamorphs emerging (Richter *et al*., 2003), there is a general relationship between fitness and both size and age at metamorphosis (reviewed by Altwegg and Reyer, 2003). Individuals metamorphosing at a large size have an increased chance of survival during the following terrestrial stage. They also grow faster and are larger at maturity than individuals metamorphosing at smaller sizes supporting the assumption that size at metamorphosis positively relates to future fitness. Similarly, age at metamorphosis is an important life history trait associated with survival probability. It is dependant on growth rate and shows high variability in amphibian populations from different environments (Merilä *et al*., 2000, 2004). Hence, the

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fitness of anuran larvae is of much interest for understanding patterns of reproductive success in wild populations, as differences in size and/or age at metamorphosis persist throughout the entire lifespan of an individual (Berven and Gill, 1983; Smith, 1987).

Variation in maternal effects is conventionally considered to be the main factor driving egg size variation (Kaplan, 1998; Laugen *et al*., 2002; Räsänen *et al*., 2005). Maternal effects could thus provide a process of transgenerational phenotypic plasticity, where the environment experienced by the mother indirectly determines the phenotype of her offspring (Mousseau and Fox, 1998). Consequently, phenotypic variation in female traits has been put forward as the major component of offspring quality (Roff, 1992). However, beyond the effects of alleles inherited, genetic variability of the parents, in general, and the mother, in particular, may play a role in determining offspring fitness (McAlpine, 1993).

The aim of our study was to explore the relationship between genetic variability and fitness in the wild as well as to investigate the possible relationship between parental genetic variation and offspring life history trait variation in two *Rana temporaria* populations in northern Finland. Under the assumption that (1) genetic variability in microsatellite loci reflects variability in genome-wide heterozygosity as postulated by the general effect hypothesis (cf. Lesbarrères *et al*., 2005), and that (2) size and age at metamorphosis are positively related to fitness, we predicted a positive relationship between these two metamorph life history traits and genetic variability.

Methods

The study species and populations

The common frog (*R. temporaria*) is the most widely distributed amphibian species in Europe (Gasc *et al*., 1997) and is a typical explosive breeder (*sensu* Wells, 1977). The spawning period lasts about 20 days, although the majority of the eggs are laid during the first few days of the breeding season (Haapanen, 1982). Both larval and adult life history traits display extensive geographic variation (Fog *et al*., 1997; Miaud *et al*., 1999; Miaud and Merilä, 2001). In northern Finland, the larval development from hatching to metamorphosis ranges from 63 to 113 days (Koskela, 1973) and local adaptation to shorter growth season in the north, as compared to more southern populations, has been suggested (Merilä *et al*., 2000).

The Kilpisjärvi study area lies within a mountainous landscape in a border zone between maritime and continental climates in northern Finland (69° 03′ N, 20° 47′ E). Kilpisjärvi is one of the coldest continental European locations in regards to the annual mean temperature (−2.3°C). The area is covered in snow from mid-October to mid-June. The duration of the growth season in the last 50 years has varied from 79 to 127 days (mean 101.12 ± 12.12 days; Järvinen, 1987; Kilpisjärvi biological station, unpublished data). In 1999, when the study was conducted, the growing season was 89 days long. Two ponds which differ in several environmental characteristics were sampled. The first pond (hereafter pond T) is rather small (530 m²) with a maximum depth of ca. 60 cm. It is situated on the edge of a subarctic open marsh which usually dries out during hot and arid summers. The second pond (hereafter pond P, 220 m² surface area, 1.6 m depth) is situated on the edge of the same marsh (the distance between the two ponds is 520 m) and is much cooler than the pond T because of the presence of ground water.

Ecological data

Continuous trapping, initiated before the emergence of frogs from hibernation, allowed the collection of all adult frogs returning to their breeding ponds. Daily monitoring of the breeding sites (4–8 visits pond^{-1} day^{-1}) allowed detection of the arrival and departure of frogs at the ponds. All captured individuals were sexed according to secondary sexual characters and morphology; weight (to nearest g) and snout-to-vent length (SVL; to nearest 0.5 mm) were also measured. Thereafter, the traps were checked twice a day until the ponds were covered with ice. Daily monitoring allowed us to determine the exact day when a given metamorph emerged from a pond. In addition, the metamorphs were weighed to the nearest 0.001 g and a complete toe was excised under anaesthesia and immediately frozen at −20°C for the purpose of the genetic analyses. A toe tip was also taken for genetic analyses from all adults captured. From mid-May to the end of September 1999, 423 offspring and 140 adults were collected by drift fencing at the two ponds. The sampling was completed by 19 September 1999 after which no metamorphs emerged. By this date, however, all the adults had not yet left the pond and the sample size for the departure date variable was thus small.

Genetic data

Both adults and metamorphs were analyzed at eight polymorphic microsatellite loci: Rt2Ca2–22 and Rt2Ca25 (T Garner, unpublished data; Lesbarrères *et al*., 2005), RRD590 (Vos *et al*., 2001), RtμH (Pidancier *et al*., 2002), RtSB03 (Berlin *et al*., 2000), Rtempμ4, Rtempμ5 and Rtempμ7 (Rowe and Beebee, 2001b). DNA extraction, polymerase chain reaction amplifications and gel electrophoresis were performed as described by Palo *et al*. (2003).

We used the program MICROCHECKER (van Oosterhout *et al*., 2004) to test for null alleles and erroneous genotyping due to stuttering. A total of 301 metamorphs were assigned to potential parents (26 males and 52 females) using the program PAPA, which assigns offspring to the most likely parental pair (likelihood approach) and allows for genotyping errors and mutations as reason for alterations of the genotype of the offspring relative to potential parents (Duchesne *et al*., 2002). To correct for errors resulting from miss-genotyping and mutations, we set the global level of transmission error to 0.01, meaning that 1% of all single-locus genotypes of the analyzed offspring might show genotyping errors or mutations; and the error distribution to 10, delimiting the assignment of mismatching offspring genotypes to one category up and down (strict stepwise model) relative to the parental genotype.

Observed heterozygosity was used as an estimate of intraindividual genetic variability in the study. This was calculated as the number of heterozygous microsatellite

loci observed for an individual divided by the total number of loci genotyped for that individual. Only individuals for which at least six loci were successfully genotyped were included in the study.

Analyses

Two offspring variables were investigated in this study: the day of departure from the pond, and their weight on this date. For each dependent variable, a linear model was used to assess the importance of phenotypic versus genetic variables. For the weight at metamorphosis, the explanatory continuous variables were the genetic variability (observed heterozygosity) of the offspring (H_O), the sire (H_S) and the dam (H_D), the size of both the sire (SVL_S) and the dam (SVL_D), the weight of both the sire (W_S) and the dam (W_D) and the pond they originated from (Pond) as a classification variable. For the day the metamorphs were captured, the explanatory continuous variables were H_O, H_S, H_D, the arrival day of both the sire (A_S) and the dam (A_D), the departure day of both the sire (D_S) and the dam (D_D), the offspring weight (W_O) and the pond they originated from (Pond) as a classification variable.

We assessed the effects of each explanatory variable based on Type III sums of squares, which reflect the influence of a variable after all other explanatory variables in the model have been accounted for (Sokal and Rohlf, 1995). For the analysis of the offspring weight, an analysis of variance (ANOVA) was used to further explore the importance of genetic variables by comparing the full model to a model including only SVL_S, SVL_D, W_S, W_D and Pond. All statistical analyses were performed with R statistical package (R Development Core Team, 2003).

Results

Both within and among ponds we captured significantly longer and heavier females (among ponds: ANOVA: $F_{1,136}=32.61$, $P<0.001$ and $F_{1,136}=53.62$, $P<0.001$ for SVL and weight, respectively; Table 1). Among both ponds, males and females arrived at the same time ($F_{1,136}=0.43$, $P=0.51$; Table 1) but females left significantly earlier than males ($F_{1,84}=6.12$, $P=0.02$; Table 1). We did not observe any difference in genetic variability among sexes ($F_{1,136}=0.002$, $P=0.97$; Table 1) nor among ponds (pond T, $H=0.56\pm0.16$; pond P, $H=0.60\pm0.17$; ANOVA: $F_{1,83}=2.83$, $P=0.1$).

Both the mean offspring weight and the mean day of capture differed among ponds. The day of capture on pond T was significantly earlier than on pond P (ANOVA: $F_{1,299}=53.74$, $P<0.001$; Table 2) and the mean offspring weight was significantly higher on pond T than on pond P (ANOVA: $F_{1,298}=10.51$, $P=0.001$; Table 2). However, there was no significant correlation between the offspring weight and the day of capture in either of the ponds (pond P: $r=-0.07$, df = 17, $P=0.782$; pond T: $r=-0.07$, df = 279, $P=0.24$).

Offspring weight was significantly influenced by the pond of origin (ANOVA: $F_{1,291}=10.9$, $P=0.001$; Table 3), offspring heterozygosity (ANOVA: $F_{1,291}=13.33$, $P<0.001$; Table 2, Figure 1) and to a lesser extent by dam heterozygosity (ANOVA: $F_{1,291}=3.98$, $P=0.047$; Table 3). Additionally, there was a significant difference between the full model and the model without the

Table 1 Descriptive information and one-way ANOVA for *Rana temporaria* adult traits in two different ponds in northern Finland

Trait	*Pond*	*Males*	*Females*	*df*	*F*
Weight	P	37.12 (6.99)	46.61 (7.5)	1,78	28.68***
	T	39.77 (8.42)	46.13 (8.2)	1,56	6.32*
	P and T	38.07 (7.53)	46.4 (7.78)	1,136	32.61***
SVL	P	70.8 (3.75)	77.1 (3.87)	1,78	46.53***
	T	71.21 (4.28)	75.88 (4.35)	1,56	12.33***
	P and T	70.95 (3.89)	76.56 (4.11)	1,136	53.62***
Arrival[a]	P	5.79 (3.11)	8.78 (4.47)	1,77	1.78
	T	9.67 (11.23)	6.95 (4.47)	1,57	8.84**[b]
	P and T	7.28 (7.48)	7.97 (4.54)	1,136	0.43
Departure[a]	P	19.89 (10.16)	14.77 (7.26)	1,60	5.1*
	T	17.38 (11.65)	13.25 (7.08)	1,22	1.17
	P and T	19.15 (10.46)	14.36 (7.18)	1,84	6.12*
H	P	0.59 (0.22)	0.61 (0.14)	1,77	0.14
	T	0.57 (0.14)	0.55 (0.16)	1,57	0.07
	P and T	0.58 (0.19)	0.58 (0.15)	1,136	0.00

Abbreviation: df, degree of freedom.
Weight (g) = mean (±s.e.) weight of males/females; SVL (mm) = mean (±s.e.) size of males/females; arrival = mean (±s.e.) arrival day of males/females; departure = mean (±s.e.) departure day of males/females; *H* = mean (±s.e.) genetic variability of males/females.
*$P<0.05$, **$P<0.01$, ***$P<0.001$.
[a]27 May 1999 was considered as day 0, as it was the date of the first adult captured.
[b]Not significant when one late male arrived on day 36 was removed.

Table 2 Descriptive information about *Rana temporaria* offspring in two different ponds in northern Finland

Pond	N_O	W_O *(g)*	*Day*	H_O	N_S	N_D
P	19	0.32 (0.05)	106.32 (6.29)	0.63 (0.16)	10 (25)	12 (55)
T	282	0.36 (0.03)	88.39 (8.91)	0.57 (0.16)	16 (16)	40 (44)

N_O = number of metamorphs; W_O = mean (±s.e.) offspring weight; day = mean (±s.e.) number of days elapsed between the beginning of the study and capture day of the metamorphs; H_O = mean (±s.e.) offspring genetic variability; N_S = number of sires assigned (total number of males); N_D = number of dams assigned (total number of females).

Table 3 Linear models of *Rana temporaria* offspring weight accounting for effects of genetic variability of the offspring (H_O), the sire (H_S) and the dam (H_D), the size of both the sire (SVL_S) and the dam (SVL_D), the weight of both the sire (W_S) and the dam (W_D) and the pond they originated from (Pond)

Source	*df*	*MS*	*F*
H_O	1	0.025	13.33***
H_S	1	0.001	0.45
H_D	1	0.007	3.98*
SVL_S	1	0.001	0.60
SVL_D	1	0.000	0.08
W_S	1	0.000	0.10
W_D	1	0.000	0.17
Pond	1	0.020	10.90**
Model	8		3.70***
Error	291	0.002	
r^2		0.09	

Abbreviations: df, degree of freedom; MS, mean square; r^2, coefficient of determination.
*$P<0.05$, **$P<0.01$, ***$P<0.001$.

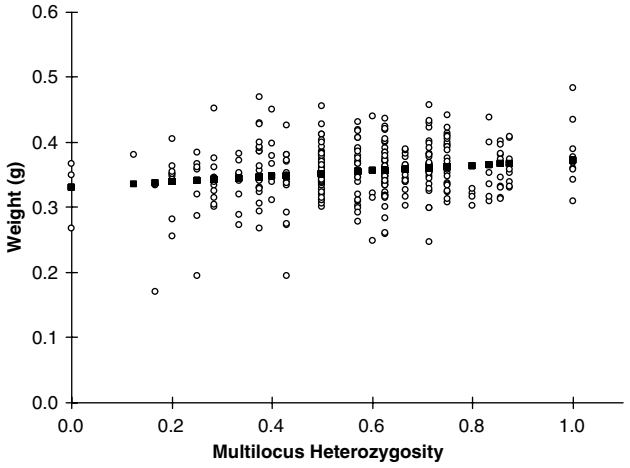

Figure 1 Predicted (filled squares) and observed (open circles) weight as a function of offspring multilocus heterozygosity in *R. temporaria* (multiple $r = 0.19$, $P < 0.001$, $n = 301$).

Table 4 Linear model of *Rana temporaria* offspring day of capture, from two populations accounting for effects of genetic variability of both the offspring (H_O), the sire (H_S) and the dam (H_D), the arrival day of both the sire (A_S) and the dam (A_D), the departure day of both the sire (D_S) and the dam (D_D), the offspring weight (W_O) and the pond they originated from (Pond)

Source	*df*	*MS*	*F*
H_O	1	128.49	1.07
H_S	1	81.61	0.68
H_D	1	19.53	0.16
A_S	1	2.81	0.02
A_D	1	0.06	0.00
D_S	1	4.99	0.04
D_D	1	516.63	4.29*
W_O	1	0.19	0.00
Pond	1	1854.07	15.39***
Model	9		2.41*
Error	23	120.46	
r^2		0.49	

Abbreviations: df, degree of freedom; MS, mean square; r^2, coefficient of determination.
*$P < 0.05$, ***$P < 0.001$.

genetic variables (i.e., H_O, H_S and H_D). The full model explained more of the variation than the reduced model (ANOVA: $F_{3,291} = 5.61$, $P < 0.001$).

The day the metamorphs were captured was well explained by the pond of origin (ANOVA: $F_{1,23} = 15.39$, $P < 0.001$; Table 4), but there was also a significant positive association with the day of departure of the dam (ANOVA: $F_{1,23} = 4.29$, $P = 0.05$; Table 3). The other possible variables did not explain variation in capture date (Table 4).

Discussion

The most salient result of this study was the positive correlation between genetic variability and offspring weight in the wild. Our results indicate that genetic variability is a reliable predictor of offspring size at the beginning of metamorph terrestrial life. This is consistent with previous results from an experimental study showing that the probability to survive until metamorphosis was higher for tadpoles with high genetic variability (Lesbarrères *et al*., 2005). While both simulation (Balloux *et al*., 2004) and empirical studies (Slate *et al*., 2004) imply that the systems and handful set of markers we currently use to study GFCs should lack statistical power and may be biased, our results support the importance of inbreeding effects. First, it is unlikely that the number of inbred individuals has been artificially increased by a non-random sampling, since it is likely that our trapping method captured most of the individuals. Second, we used the same panel of markers in a previous study where the hypothesis of linkage disequilibrium was discarded (Lesbarrères *et al*., 2005). Therefore, it is unlikely that the association between genetic variability and offspring weight at metamorphosis was observed purely by chance.

Another significant result was the effect of genetic variability of the dam on offspring emergence from the pond. Maternal effects on offspring traits in amphibians are usually explained by differential investment in egg number and size (Kaplan, 1998; Laugen *et al*., 2002; Pakkasmaa *et al*., 2003; Räsänen *et al*., 2005). Our results suggest the possibility that more heterozygous females are better at nourishing their eggs with yolk as compared with less heterozygous females (Danzmann *et al*., 1989). Further studies would be needed to investigate this relationship. An alternative possibility is that this association is a result of the non-independence of parent–offspring heterozygosity estimates (Mitton *et al*., 1993). Conversely, while previous studies have found genetic effects to be important determinants of growth rate, age and size at metamorphosis (e.g., Laugen *et al*., 2002), we did not observe any effect of male genetic variability.

We did not find any association between genetic variability and offspring capture date. Assuming that larval development is fairly constant within a pond, we propose that females leave the pond just after they have spawned and that environmental forces within the pond then drive the development of the larvae until dispersal. Similarly, in a previous common garden experiment, a lack of adaptive response to desiccation risk in northern larvae was observed (Laurila *et al*., 2002). Owing to the combined effects of water temperature and short growing season, this further suggests that environmental selection is strong enough to override genetic effects on developmental rates (Laugen *et al*., 2003) and favours rapid development in northern populations at the expense of phenotypic plasticity. Therefore, natural selection is likely to be the primary agent driving larval development and, although we used the offspring capture date as a proxy for time to metamorphosis, our results suggest that a more proximal cause of this trait variation might be the departure day of the female. In the trade-off between size and age at metamorphosis, there remains controversy regarding the possible correlation between these two traits (i.e., Gibbons and McCarthy, 1986; Miaud *et al*., 1999; Merilä *et al*., 2000). Similar to our results, size at metamorphosis was not related to timing of metamorphosis in an 8-year-study of *R. temporaria* (Loman, 2002). While timing of metamorphosis was influenced by temperature and dry-out events, size at metamorphosis was found to be influenced mostly by density effects (Loman, 2002). We suggest that size at

metamorphosis is more important than time to metamorphosis in environments with strong selection pressure on the time to metamorphose (Merilä *et al*., 2004), hereby confirming the experimental results of Altwegg and Reyer (2003).

Our results also confirm the environmental dependency of early life history traits, since the pond effect significantly explained variation in both studied traits. This shows that local environment variation is important for tadpole development (Loman, 2002; Laugen *et al*., 2003), and that evolution of interpopulational differences in plastic responses is possible (Laurila *et al*., 2002). Pond P froze over for a short period after the first spawning had taken place, which prevented a large number of eggs from successfully hatching. Also, owing to lower water temperature in pond P as compared to pond T more tadpoles never finished metamorphosing. While the relationship between larval fitness trait variation investigated under controlled laboratory conditions and that seen at presumed-neutral microsatellite loci is not always observed (Rowe and Beebee, 2001a), our study identified such a correlation in the wild. Hence, our results suggest a disadvantage for homozygous individuals, particularly during stressful conditions (Crnokrak and Roff, 1999; Rowe and Beebee, 2003; Lesbarrères *et al*., 2005; Pearman and Garner, 2005).

Taken together, our results suggest that genetic variability is associated with offspring size at metamorphosis in *R. temporaria* with a disadvantage for homozygous individuals. Furthermore, this study revealed that dam genetic variability is associated with offspring size, which in turn determines fitness, as there is a higher probability of survival for bigger offspring. Finally, despite the constant debate over the genetic basis of GFCs, we suggest that amphibian populations provide good candidates to study fitness variation due to genome-wide heterozygosity.

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