SHORT COMMUNICATION

**Attila Hettyey · Anssi Laurila · Gábor Herczeg ·
K. Ingemar Jönsson · Tibor Kovács · Juha Merilä**

Does testis weight decline towards the Subarctic? A case study on the common frog, *Rana temporaria*

Abstract Interpopulation comparisons of variation in resource availability and in allocation patterns along altitudinal and latitudinal gradients allow insights into the mechanisms shaping the life history of animals. Patterns of between-population differences in female life history traits have been studied intensively across a wide range of taxa, but similar investigations in males have remained scarce. To study if testis weight—a measure of reproductive investment—varies on a geographical scale in anurans, we focussed on the variation in relative testis weight (RelTW) and asymmetry in 22 populations of the common frog *Rana temporaria* along a 1,600-km latitudinal transect across the Scandinavian peninsula. We found that RelTW decreased towards the north. Body mass and body length both had independent positive effects on testes mass. We found evidence for directional asymmetry (DA) in testis weight with the right testis being larger than the left. The level of DA in testis weight was not related to latitude, but both body mass and testes mass had independent positive effects on asymmetry. We discuss the northwards decrease in RelTW in terms of a decreased reproductive investment as a possi-
ble consequence of harsher environmental conditions, and perhaps also, weaker sexual selection in the north than in the south.

Introduction

Life history theory predicts a trade-off between current reproductive effort and future reproductive success and/or survival (Lack 1966). The fecundity cost, i.e. a negative relationship between current allocation to reproduction and future fecundity, and the survival cost, i.e. a decreasing probability of survival with increasing current reproductive effort, both originate from resource limitation. A fixed resource budget makes it inevitable that there are trade-offs for allocation to growth, maintenance and reproduction. There is increasing evidence that patterns of allocation to these life history traits show between-population variation due to local adaptation and environmentally induced phenotypic differences (e.g. Berven 1982; Lüddecke 2002; Laugen et al. 2003). Recent work, however, has focussed mainly on female life history trait variation and much less is known about variation in males.

We studied variation in relative testis weight (RelTW) in the common frog, *Rana temporaria*, along a latitudinal gradient ranging from southern Sweden to northern Finland. Testis weight relative to body weight (BW) is widely used as a measure of reproductive investment in males and is regarded to stand under strong sexual selection (e.g. Byrne et al. 2002). Our prediction was that RelTW would decrease towards the north for two reasons. Firstly, possibilities for energy acquisition decrease strongly due to a drastically shortened activity season (Laugen et al. 2003) possibly allowing relatively less energy to be allocated to reproduction in the north than in the south. Secondly, the operational sex ratio (OSR) changes with low female/male ratios in the south (e.g. Arak 1982; Ryser 1986) towards an OSR exceeding unity in the north (Elmberg 1990; J. Merilä, unpublished data) resulting in lowered intrasexual competition in males, both at the level of social interactions and at the level of sperm competition.

A. Hettyey (✉) · G. Herczeg · T. Kovács
Behavioural Ecology Group, Department of Systematic
Zoology and Ecology, Eötvös Loránd University,
Budapest, 1117 Pázmány Péter s. 1/c, Hungary
e-mail: hettyeya@freemail.hu
Tel.: +3612090555
Fax: +3613812194

A. Laurila
Department of Population Biology, Evolutionary Biology
Centre, Uppsala University,
Norbyvägen 18 d, 75236 Uppsala, Sweden

K. I. Jönsson
Department of Theoretical Ecology, Lund University, Ecology
Building,
22362 Lund, Sweden

J. Merilä
Ecological Genetics Research Unit, Department of Biological
and Environmental Sciences, University of Helsinki,
P.O. Box 65, 00014 Finland

Another, distinct aim of our study was to explore asymmetry in testes and potential latitudinal trends in asymmetry patterns. It has been proposed by Møller (1994) that in testis weight directional asymmetry (DA) should be the norm and that only males in good condition may be able to develop large degrees of DA. We predicted DA to occur in our samples in all studied populations, and the level of DA to be positively correlated with body condition. As time available for growth and development decreases strongly towards the north (Elmberg and Lundberg 1991; Laugen et al. 2003) and heterozygosity is expected to decrease towards the edge of a species' distribution area (Rowe et al. 1999), we expected a negative correlation between the level of DA and latitude as a consequence of increasing developmental stress (sensu Møller 1994).

Materials and methods

Study species

The common frog (*Rana temporaria* Linnaeus 1758) is a large (54–78 mm in our samples), widespread anuran native to the Palaearctic region. It ranges from the southern Balkans to subarctic Fennoscandia. It is an explosive breeder (sensu Wells 1977) males actively search and fight for females, mating and egg-deposition occur on aggregated leks and the breeding season lasts 7–24 days (Elmberg 1990). Sperm competition has been proposed to be common in *R. temporaria* (Laurila and Seppä 1998), consequently, sperm competitiveness and thus testis weight may be an important determinant of male reproductive success. Males and females lose around 30% and 50% of their total BW, respectively, during spawning (Ryser 1989) implying that reproduction has a high energetic cost in this species.

Data collection

In 1998–2002, we collected adult male common frogs from 22 localities along a 1,600-km latitudinal transect (Fig. 1). To ensure that individuals are sampled at the same time in their breeding cycle we performed sampling during the early spawning period at each locality. Frogs were hand-collected from the breeding ponds. Collected frogs were kept in water at about 6°C for 2 h to 4 days until they were anaesthetised with MS-222, dissected and measured. From each individual, we measured snout-vent length (SVL; to the nearest 0.1 mm) with digital callipers and BW (to the nearest 0.1 g) with a Pesola spring balance. Right and left testes were weighed separately [right testis weight (RTW) and left testis weight (LTW); to the nearest 0.001 g] with an electronic balance. RelTW was defined as the sum of the weights of the two testes.

Statistical analyses

LTW, RTW and RelTW were $\log_{10}$-transformed to achieve normality and enhance homogeneity of variances. For
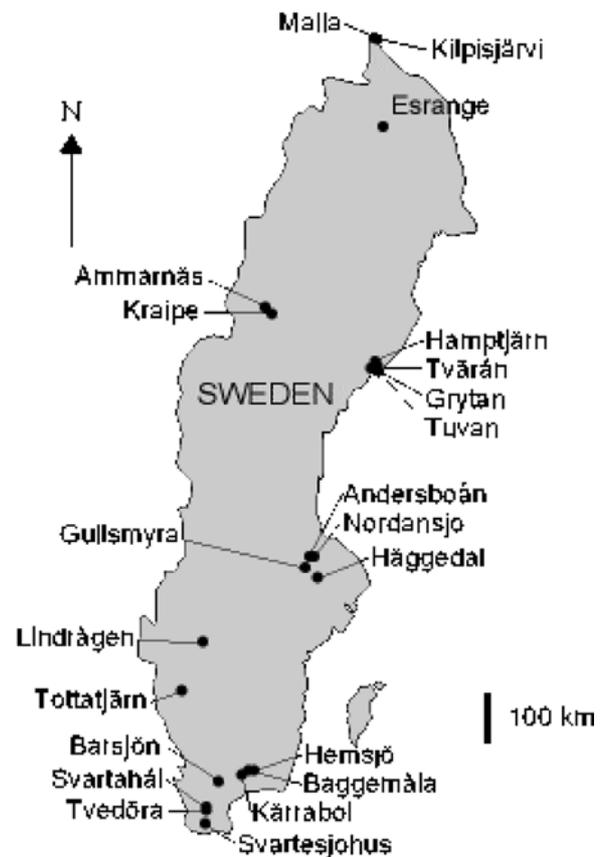
Fig. 1 Map showing the locations of the study populations

65 males, only total weight of the two testes was measured, and these data were excluded from the asymmetry analyses. Similarly, BW was not measured for eight males, and these data were excluded from the analyses using BW as a predictor variable.

We used mixed GLM in PROC MIXED of SAS (Littell et al. 1996) to test latitudinal (both linear and quadratic) and altitudinal patterns of variation in testis weight together with SVL and BW. Population was fitted in the model as a random effect, whereas standardized values of the other variables were fitted as covariates. We first tested for DA in testis weight by performing a repeated-measures ANOVA, in which left and right testes were treated as a repeated measure of the same subject and population was entered as a random factor. This procedure allowed us to account for possible variation due to population origin. We then used mixed GLM again to analyse the geographical pattern in DA (measured as RTW–LTW).

Results

Testis weight

Mixed GLM showed that while population effect was significant ($Z=2.32$, $P=0.020$), increasing latitude had a negative effect on RelTW ($B=-2.788$, $F_{1,21.9}=15.38$, $P<0.001$, Fig. 2a). Altitude had a negative, although

Fig. 2 Relationship between relative testis weight and **a** latitude, **b** snout–vent length (*SVL*) and **c** body mass. Both testis weight and predictor variables are presented as residual values from mixed GLMs. For ease of interpretation only population mean values are shown. The *regression lines* are based on population mean values and are shown only for illustrative purposes. Note that the apparent *outliers* are usually small populations and their removal does not affect the results
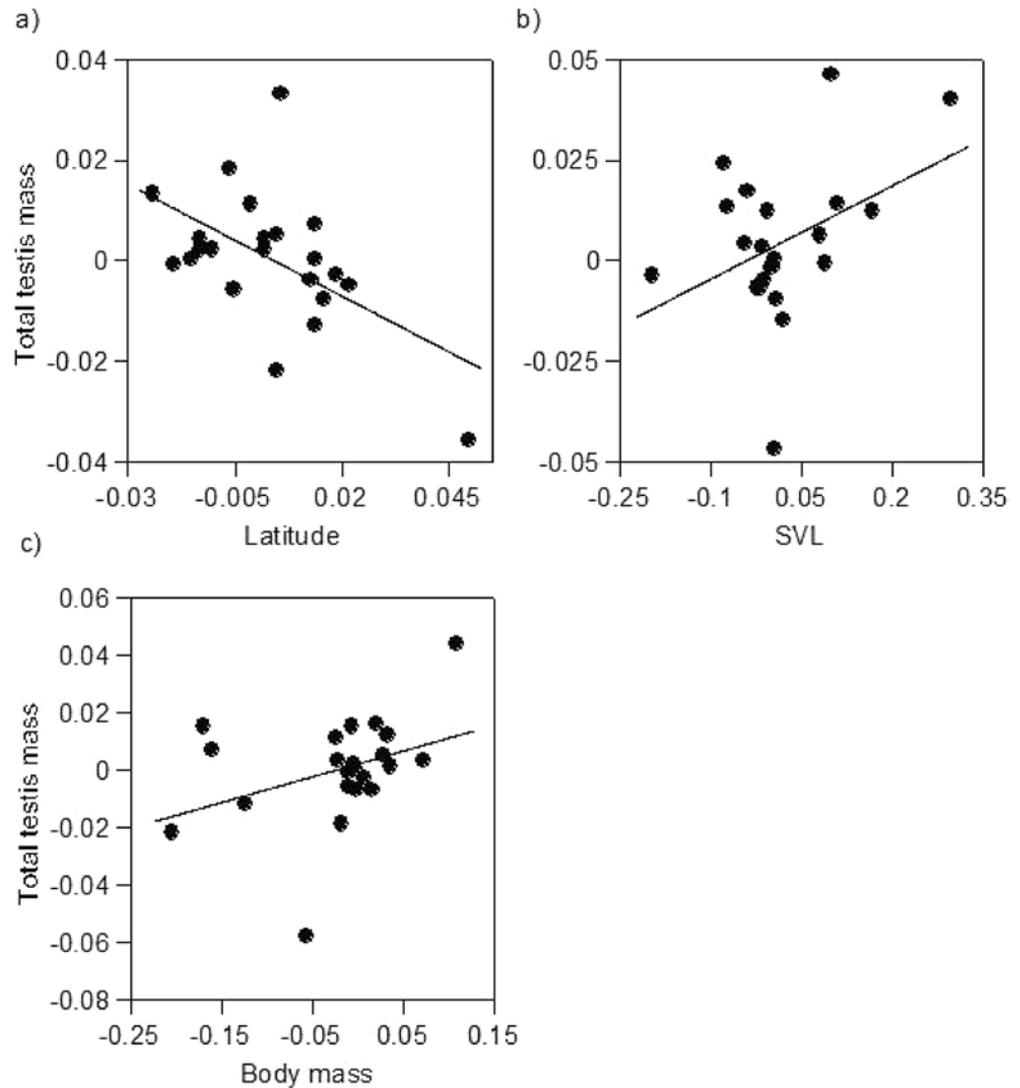
non-significant effect ($B=-0.056$, $F_{1,23.1}=3.77$, $P=0.065$). Both SVL ($B=0.035$, $F_{1,577}=8.42$, $P=0.004$) and BW ($B=0.140$, $F_{1,576}=133.90$, $P<0.001$) affected RelTW positively (Fig. 2b and c).

Testis asymmetry

We found evidence for DA in testis weight as indicated by the significant testis within-subjects effect (repeated-measures ANOVA, $F_1=7.54$, $P<0.001$). In 20 out of 22 populations the right testis was bigger than the left one. The significant population between-subjects effect again indicated that populations differed in testis weight (repeated-measures ANOVA, $F_{21}=5.98$, $P<0.001$). Mixed GLM indicated that when BW and RelTW were accounted for, the populations did not differ in DA, neither were there any latitudinal or altitudinal effects ($P>0.31$). Instead, we found that both BW ($B=0.003$, $F_{1,239}=5.82$, $P=0.017$) and RelTW ($B=0.003$, $F_{1,186}=8.72$, $P=0.004$) had significant positive effects on DA: heavier *R. temporaria* males and

those with larger testes had stronger DA (relatively larger right testis; Fig. 3). However, SVL did not affect DA ($F_{1,207}=0.73$, $P=0.395$).

Discussion

Since the founding papers of Short (1979) and Harcourt et al. (1981), interspecific comparisons of testis weight have become a commonplace interest in evolutionary ecology. The main focus of explanations for the evolution of testis weight has been on the variation due to differences in the breeding system, and the resultant variation in risk and intensity of sperm competition (e.g. Byrne et al. 2002). However, Emerson (1997) pointed out that factors other than sperm competition, such as variation in female fecundity or male–male agonistic behaviour can have significant consequences on RelTW and have to be considered when interpreting patterns of testis weight. This should apply to intraspecific comparisons between populations as well.

Fig. 3 Relationship between directional testis asymmetry and **a** body mass, **b** total testis mass. Testis asymmetry is presented as residual values from mixed GLMs. For ease of interpretation population means are shown. The *regression lines* are based on population mean values and are shown only for illustrative purposes
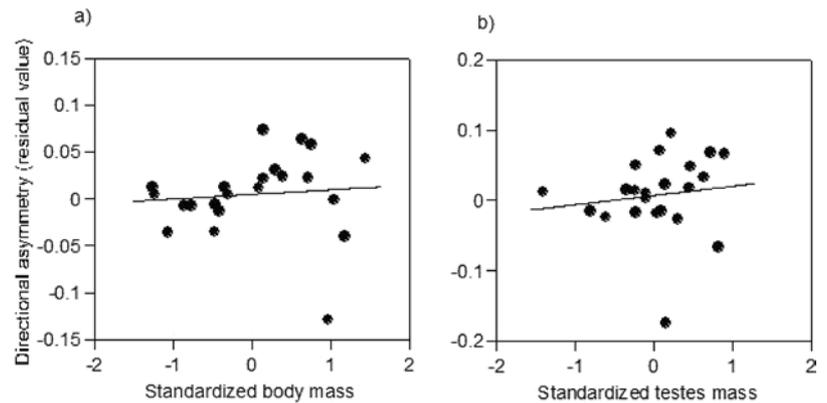
The observed negative relationship between latitude and RelTW is most probably a result of a combination of different factors. Some of the variation in RelTW may be due to extreme differences in the time available for reproduction and for the re-acquisition of resources needed for survival. The difference in the length of the activity period between southern Sweden and northern Finland (6 and 3 months, respectively; see Elmberg and Lundberg 1991; Laugen et al. 2003) might differentially constrain the resource budget, potentially resulting in a gradual shift in energy allocation patterns parallel to increasing latitude (see also Elmberg and Lundberg 1991). This hypothesis is supported by a similarly decreasing allocation to reproduction in female common frogs at high latitudes (cf. Koskela and Pasanen 1975 and Gibbons and McCarthy 1986; or see Hönig 1966; Haapanen 1982).

Variation in the strength of sexual selection may also cause differences in testis weight among populations. In populations where OSR is high, males do not have to compete intensively for females (Wells 1977). If this is the case, the level of aggression and testosterone can be low with smaller testis weight as a possible result. Similarly, the risk and intensity of sperm competition may be lower in populations with higher OSRs. As sperm competition sensu Parker (1970) is predicted to select for increased sperm production and for increased testis weight (Harcourt et al. 1981), we might expect larger testis weights in populations with more skewed OSRs. Increasing OSRs towards the north may have contributed to the latitudinal decline in RelTW.

Our results on testis asymmetry are consistent with Møller's (1994) hypothesis stating that DA in testis weight represents the optimal phenotype and thus should be the norm. Testis asymmetry was positively correlated with body mass but not with SVL. This may be understood as the development of asymmetry in testes being adaptive but incurring high costs, probably by causing physiological or morphological handicaps that only individuals in good condition can cope with. Deviations from DA have been proposed as a measure of developmental stress (Møller 1994). Palo et al. (2003) found no evidence for a latitudinal trend in genetic stress within the common frog populations included in this study, so that the genetic component of developmental stress is likely to equal zero. However, as we found

no correlation between latitude and the level of DA despite great differences in environmental conditions and presumably environmental stress (cf. Elmberg and Lundberg 1991; Laugen et al. 2003), our data do not support DA in testes as a reliable measure of developmental stress.

In summary, we detected systematic between-population differences in RelTW on a geographical scale and offer hypotheses that could explain the observed pattern. Further, we found DA in testes to be the main component of asymmetry over the whole range of our investigation and that the level of asymmetry was condition-dependent. However, reliable evolutionary interpretations of life history and asymmetry patterns require a distinction between genotypic variation and environmentally induced phenotypic variation (Berven 1982). Our results thus represent a good basis for future experimental work seeking verification for mechanisms shaping life history in animals.

References

Arak A (1982) Male–male competition and mate choice in frogs and toads. PhD thesis. University of Cambridge, Cambridge

Berven KA (1982) The genetic basis of altitudinal variation in the wood frog *Rana sylvatica*. I. An experimental analysis of life history traits. Evolution 36:962–983

Byrne P, Roberts JD, Simmons LW (2002) Sperm competition selects for increased testis mass in Australian frogs. J Evol Biol 15:347–355

Emerson SB (1997) Testis size variation in frogs: testing the alternatives. Behav Ecol Sociobiol 41:227–235

Elmberg J (1990) Long-term survival, length of the breeding season, and operational sex ratio in a boreal population of common frogs *Rana temporaria* L. Can J Zool 68:121–127

Elmberg J, Lundberg P (1991) Intraspecific variation in calling, time allocation and energy reserves in breeding male common frogs *Rana temporaria*. Ann Zool Fenn 28:23–29

Gibbons MM, McCarthy TK (1986) The reproductive output of frogs *Rana temporaria* (L.) with particular reference to body size and age. J Zool 209:579–593

Haapanen A (1982) Breeding of the common frog (*Rana temporaria* L.). Ann Zool Fenn 19:75–79

Harcourt AH, Harvey PH, Larson SG, Short RV (1981) Testis weight, body weight and breeding system in primates. Nature 293:55–57

Hönig J (1966) Über Eizahlen von *Rana temporaria*. Salamandra 2:70–72

Koskela P, Pasanen S (1975) The reproductive biology of the female common frog, *Rana temporaria* L., in northern Finland. Aquilo Ser Zool 16:1–12

Lack D (1966) Population studies on birds. Clarendon, Oxford

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

Laurila A, Seppä P (1998) Multiple paternity in the common frog (*Rana temporaria*): genetic evidence from tadpole kin groups. Biol J Linn Soc 63:221–232

Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute, Cary, N.C.

Lüddecke H (2002) Variation and trade-offs in reproductive output of the Andean frog *Hyla labialis*. Oecologia 130:403–410

Møller AP (1994) Directional selection on directional asymmetry: testes size and secondary sexual characters in birds. Proc R Soc Lond Ser B 258:147–151

Palo JU, O'Hara RB, Laugen AT, Laurila A, Primmer CR, Merilä J (2003) Latitudinal divergence of common frog (*Rana temporaria*) life history traits by natural selection: evidence from a comparison of molecular and quantitative genetic data. Mol Ecol 12:1963–1978

Parker GA (1970) Sperm competition and its evolutionary consequences in insects. Biol Rev 45:525–567

Rowe G, Beebee TJC, Burke T (1999) Microsatellite heterozygosity, fitness and demography in natterjack toads *Bufo calamita*. Anim Conserv 2:85–92

Ryser J (1986) Altersstruktur, Geschlechtsverhältnis und Dynamik einer Grasfrosch-Population (*Rana temporaria* L.) aus der Schweiz. Zool Anz 217:234–251

Ryser J (1989) Weight loss, reproductive output, and the cost of reproduction in the common frog, *Rana temporaria*. Oecologia 78:264–268

Short RV (1979) Sexual selection and its component parts, somatic and genital selection, as illustrated by man and the great apes. Adv Stud Behav 9:131–158

Wells KD (1977) The social behaviour of anuran amphibians. Anim Behav 25:666–693