Doctoral thesis

For the degree of Doctor of Philosophy

Ecological genetics of inbreeding, outbreeding and immunocompetence in *Ranid* frogs

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Abstract Using artificial fertilization, I crossed frogs from different populations to evaluate fitness consequences for the offspring from an inbreeding-outbreeding perspective, and to evaluate quantitative genetic effects on immunocompetence against a fungal pathogen (*Saprolegnia*). Crosses between closely situated populations of different sizes generated contrasting results for the effects of outbreeding on offspring traits between populations and life history stages, emphasizing the importance of epistatic effects and the difficulties of relying on generalizations when making conservation decisions (e.g., regarding translocations). Experimental infection of frog eggs from six populations with *Saprolegnia* fungus showed a significant family effect on the degree of infection of eggs and embryos, in particular at lower fertilization success and with a significant temperature × population interaction effect. A paternal genetic effect on fungus resistance was found using a half-sib split design. Furthermore, relatively more eggs were infected when fertilized by sperm from the same, in contrast with a different population. However, there was no evidence for a stronger effect in isolated island populations. Although the mechanistic underpinnings remain unknown, these results suggest substantial levels of genetic variation in resistance to *Saprolegnia* in natural populations within and among populations. We also found that pre-hatching exposure to *Saprolegnia* dramatically reduced the size at metamorphosis in the absence of further exposure to the fungus, possible as a delayed effect of impaired embryonic development. However, in contrast to some other amphibians, induced hatching in response to *Saprolegnia* could not be confirmed. In conclusion, the results suggest that frog populations are genetically diverse even at small geographic scale with frequently strong and unpredictable consequences of in- and outbreeding for the response to stressors.

List of papers

This thesis is based on the following papers, which are referred to by their roman numerals:

I. Sagvik, J., Uller, T. & Olsson, M. 2005. Outbreeding depression in the Common frog, *Rana temporaria*. *Conservation Genetics*. 6: 205-211.

II. Uller, T., Sagvik, J. & Olsson, M. 2006. Crosses between frog populations reveal genetic divergence in larval life history at short geographic distance. *Biological Journal of the Linnean Society*. 89: 189-195.

III. Sagvik, J., Uller, T., Stenlund, T. & Olsson, M. 2008. Intraspecific variation in resistance of frog eggs to fungal infection. *Evolutionary Ecology*. 22: 193-201.

IV. Sagvik, J., Uller, T. & Olsson, M. 2008. A genetic component of resistance to fungal infection in frog embryos. *Proceedings of the Royal Society of London, B*. 275: 1393-1396.

V. Uller, T., Sagvik, J. & Olsson, M. 2008. Pre-hatching exposure to water mold reduces size at metamorphosis in the moor frog. *Submitted*.

Contents

Introduction

Down, July, 1870

'My Dear Lubbock,

It is manifestly desirable that…the truth…that consanguineous marriages lead to deafness, dumbness and blindness…could easily be ascertained'.

Believe me,

Yours sincerely

Charles Darwin

Considering that Darwin corresponded with a dear friend about the importance of inbreeding some 130 years ago, we would perhaps expect that today's biologists have a thorough understanding of *how* important inbreeding (and outbreeding – its diametric counterpart) is in terms of long-term survival in the wild. But this is far from the truth. Just over a decade ago, one of the leading conservation biologists of our time claimed that there was little evidence that inbreeding contributes to extinction (Caughley 1994). He was right – simply because very few studies had addressed this problem in natural populations, in spite of being remarked on since the mid 1700's, and researched in captive populations (Kölreuter 1766; Knight 1799; Darwin 1876; Frankham 1995). Since then, a handful of studies have also shown that inbreeding depression does occur in free-ranging populations and that it may compromise long-term survival, for example in Swedish adder snakes (*Vipera berus*: Madsen et al. 1999; Frankham 1995), song sparrows (*Melospiza melodia*: Smith et al. 2006), southern dunlins (*Calidris alpina schinzii*: Blomqvist and Pauliny 2007) and Darwin's finches (*Geospiza sp.*: Keller et al. 2002), but in the latter case only under food stress.

A problem with most studies in natural populations is that any evidence relating to inbreeding and inbreeding depression becomes correlational and circumstantial; we can only passively observe simultaneous loss of genetic variation (for example in molecular genetics markers) and associated loss of organismal viability. Under some circumstances it may even be sex-specific and lead to fitness costs associated primarily with the

production of one sex offspring, such as in Swedish sand lizards (*Lacerta agilis*: Olsson et al. 2004) and Canadian song sparrows (Smith et al. 2006). My thesis specifically targets three routes to fitness erosion, namely inbreeding, outbreeding, and their potential genetic effects on immunocompetence in Swedish brown frogs (*Rana* sp).

Relatedness effects on offspring viability can be thought of as acting along an inbreeding-outbreeding continuum, with some 'optimal' relatedness of a mating pair somewhere in the middle (Thornhill 1993). When two closely related individuals mate, the causal mechanism of inbreeding depression may occur by an increase in the frequency of homozygotes and 1) associated decrease in the frequency of superior heterozygotes or 2) expression of deleterious recessive alleles; Roff 2002). Most recent work favors the latter mechanism as the most important cause of inbreeding depression (Roff 2002).

Outbreeding, on the other hand, i.e., the negative effect of siring offspring with a too distantly related partner, is believed to be the result of breaking up co-adapted gene complexes, which results in the disruption of local adaptation (Waser et al. 2000). The maximally negative effect of this can be thought of as hybridization. However, even in this case there can be positive effects long term, such as when genetic elements are back-crossed into the parental species (Arnold 1997).

Examples of the importance of inbreeding and outbreeding for shaping the natural history and mating tactics of organisms in the wild are colorful and varied. For example, a comparison of selfing and hermaphroditic nematodes (*Caenorhabditis elegans vs. remanei*) showed that the sexual species suffered greatly from inbreeding depression, whereas the selfing species suffered equally from outbreeding (Dolgin et al. 2007). In natural populations of the ornate dragon lizard (*Ctenophorus ornatus*), more outbred females produced offspring with poorer survival (LeBas 2002), and in rainbow trout (*Onchorhynchus mykiss*), crossings between wild and captive-reared fish resulted in outbreeding depression (Tymchuk et al. 2007). One of the most extreme examples from the animal kingdom may be Ambrosia beetles (*Xyleborini sp.*), which regularly mate with siblings, and in which outbreeding, but not inbreeding, results in genetic depression (Peer and Taborsky 2005).

The costs in terms of phenotypic viability at both ends of the inbreeding-outbreeding continuum suggest that selection should be stabilizing on genetic mate choice. However, Kokko and Ots (2006) rightly point out that inbreeding should not always be avoided and not at any cost. One often overlooked component in this scenario is inclusive fitness benefits from mating with a more closely related partner, in particular at limited breeding opportunities. Furthermore, when selfing leads to purging of detrimental alleles, then mating with a more closely related individual may come at a lower price in terms of offspring inviability (with maintained inclusive fitness benefits, all else equal). One such example may be mate preferences for siblings over more outbred partners in the cestode (*Schistocephalus solidus*: Schjørring and Jäger 2007).

Inbreeding can have many faces when it comes to compromised fitness. Recent work shows that immunocompetence, the ability to fight pathogens, may become severely compromised by inbreeding depression, resulting in dwindling population density and even threatened survival. An example of this is the isolated lions in the Ngoro Ngoro crater that have repeatedly been close to extinction following Tsetse fly epidemics (Frankham 1995). One of the underlying reasons for this is the rapid evolution of pathogens compared to hosts, which inevitably leads to strong selection for host counter-adaptations in an ongoing evolutionary arms race (Slev and Potts 2002). Evidence of this comes from the rapid evolution of immune system genes (Slev and Potts 2002), which requires genetic variation to proceed. Thus, a reduction in host genotype variation predicts a reduced capacity for evolutionary change, which prolongs the adaptive lag phase to the pathogen. This leads to reduced immunocapacity in response to inbreeding at the level of the individual, as shown in inbred strains of mice (Gurwitz and Weizman 2001). At a population level, however, data on pathogenic threats to long-term survival are meager, but recent work confirms potentially devastating effects on sustainability of small populations (Frankham 1995).

Most work in free-ranging populations has focused on additive genetic influences on immunity and confirmation of its heritability (Sorci et al. 1997; Smith et al. 1999; Svensson et al. 2001). However, inbreeding affects phenotypic traits through dominance rather than additive genetic effects, and dominance effects are normally not heritable in sexually reproducing organisms (Roff 1997). This predicts a different set of genetic

mechanisms for loss of immunocompetence with increased inbreeding compared to other phenotypic traits. The complexity of these mechanisms is reflected in the inconsistent relationship between inbreeding and parasite resistance across host and pathogen taxa (Cassinello et al. 2001; Arkush et al. 2002). Disentangling the importance of quantitative genetic factors from conditional ones would be greatly facilitated with model species that readily accept captive conditions, are external fertilizers, and for which artificial fertilization techniques have been developed. My study rests on experimental designs with such model systems – the Swedish brown frogs (*Rana sp*., more specifically the common frog *R. temporaria* and the moor frog, *R. arvalis*). In the next section I outline how we approach questions regarding among-population genetic heterogeneity, outcrossing, quantitative genetic and sex-specific parental effects on fungus susceptibility, using artificial breeding designs.

Material and methods

Study species and study populations

The common frog (*Rana temporaria*) and the moor frog (*Rana arvalis)* are medium sized Ranid frogs (total length of about 8-9 cm), distributed over large parts of Europe and parts of Asia (Gasc et al. 1997). Both species are common in Sweden and distributed over most parts of the country. The mating season in south-western Sweden occurs in March-April. Both species are explosive breeders, that is, most matings take place on a limited area (often a few square meters) during a few days. Since frogs have external fertilization of the eggs, the chance of multiple mating exists and has been observed (Roberts et al. 1999, Laurila and Seppä 1998, personal observation), although it does not seem to be very common. Up to five moor frog males have been observed trying to mate a single female, and males of both study species sometimes kill the female when they try to mate (personal observation). Females normally oviposit once per season, but two or more clutches are sometimes laid (Duellman and Trueb 1994). Males make several breeding attempts and stay in the breeding area longer than the females (Elmberg 1990).

We have used two common frog populations (paper I and II) and six moor frog populations in our work (paper III-V; Table 1).

Table 1. Study populations

Population	Coordinates	Population size*	N (F:M)	Isolation
Common frog				
Dingle	58°31'N, 11°34'E	50-75	22:22	Isolated, no breeding ponds within 5 km
Onsala	57°26'N, 11°59'E	>2000	22:22	Not isolated
Moor frog				
Björkö	57°44'N, 11°40'E	25-75	23:46	Isolated island
Öckerö	57°43'N, 11°38'E	25-75	23:46	Isolated island
Hisingen	57°45'N, 11°48'E	>100	14:37	Not isolated
Änggården	57°40'N, 11°57'E	>200	7:30	Not isolated
Måryd	55°42'N, 13°21'E	>100	23:46	Not isolated
Frihult	55°33'N, 13°38'E	>100	23:46	Not isolated

*Population size: based on the number of clutches found during the breeding season.

Sampling procedures

Frogs were caught by hand at night during the breeding season and transported to the Zoology Department, University of Gothenburg where they were kept in darkness at 4°C for up to ten days before the onset of the experiments. Mass of all adult frogs was measured to the nearest 0.1 g on an electronic scale.

Experimental design (general)

All experiments are based on the artificial fertilization procedure following the protocol outlined by Berger et al. (1994). In short, the hormone LHRH (Luteinizing hormone releasing hormone, Sigma Aldrich) was subcutaneously injected (100 mg/g body mass) in the flank of the frogs, which induces ovulation within 24 hours and male sperm shedding into the cloaca within an hour.

Eggs were stripped from each female by applying a gentle pressure to the abdomen, and partitioned into Petri dishes containing a sperm solution from one or more randomly chosen males (depending on experimental design). Eggs from each female were fertilized by one male from the same population and one male from another population, or a mix of two males from the same or different populations i.e., gametes were mixed in different crossing regimes (see each paper for details). In all cases, the sperm/egg mixture was immediately covered with tap water (Zoology Department, University of Gothenburg) and water was continuously replenished as it was absorbed by

the eggs. Between 10 and 20 eggs from each female were preserved in 5% formaldehyde for analysis of maternal investment (i.e., egg size). After approximately two hours, the fertilized eggs from each Petri dish were separated into plastic jars. Thereafter project-specific experimental designs were applied (see the separate chapters below).

Experimental design (common frog, paper I-II)

A pool system was arranged with two replicate pools (152 x 122 x 25 cm) per two water temperature treatments (15 and 20°C). Eighty-six one-litre plastic jars with wire mesh bottoms (to ensure water circulation) were hung from crossbars in all four pools. The four pools were set up using tap water that was aerated for a minimum of ten days before the onset of the experiments. The photoperiod was set to a 14:10 L:D regime.

Females from both populations were crossed with males from the same population and males from the other population (i.e., Dingle (D), Onsala (O); D-D, D-O, O-O, O-D). Each male was also used in two crosses, one within and one between populations.

Each jar received approximately 60 eggs from each cross, i.e. ca. 240 eggs per cross were separated into four samples, allocated to two replicates in the two temperature treatments. When the eggs had hatched (stage 23, Gosner 1960), all tadpoles except eight per jar were removed and preserved in five percent formaldehyde for subsequent measuring and scoring of malformations. Completely undeveloped eggs were classified as infertile. There was virtually no embryonic death before hatching, and fertilization success was therefore calculated as the proportion of hatched eggs. Four randomly chosen tadpoles from each replicate were measured in a stereoscope to the nearest 0.06 mm (set by the distance between ruler bars in the eye-piece of the stereoscope; we measured total length, tail length, length from snout to gill and tail width). All tadpoles were inspected for malformations, such as kinked vertebrae and malformed tails. To obtain a measure of maternal investment, we took two measurements of egg diameter from each of 12 eggs per female, to the nearest 0.06 mm using a stereoscope. The eight tadpoles per jar that were left were raised to metamorphosis (stage 42, Gosner 1960) and weighed to the nearest mg on an electronic scale after soaking up excess water with a paper towel. Total length and body length were measured to the nearest 0.5 mm using a ruler. During

growth, the eight tadpoles were fed commercially available fish food (Sera San, Heinsberg, Germany) *ad libitum* and water was cleaned with a high capacity filter.

Experimental design (moor frog, paper III-V)

We used two temperature rooms (15°C and 18°C: paper V only 18°C) and each temperature treatment differed no more than 0.2°C between jars at any given time throughout the experiments. The photoperiod was set to a 14:10 L:D regime.

Females from all six populations were crossed with males from the same and another population (paired so that crosses were made between B-M and H populations and Ö-F-Ä populations respectively; for population information see Table 1).

Each jar (filled with 0.95 litre of aerated tap water) received approximately 70 eggs. When approximately 95% of the developing embryos in a jar had hatched (stage 23, Gosner 1960), four tadpoles were preserved in 5% formaldehyde for later measurements. The rest of the eggs/embryos/tadpoles were counted and tadpoles were scored for malformations (malformed/not malformed, as per definitions stated above). Undeveloped eggs were classified as unfertilized. Four randomly selected tadpoles with no visual sign of malformations were kept in each jar and raised under the same conditions as described above. The remaining tadpoles were fed commercially available fish food (Sera San, Heinsberg, Germany) *ad libitum*. Water was changed and containers thoroughly cleaned and disinfected (Debisan, Nordex, Sweden) every third or fourth day or, from approximately two-thirds into development, every second day. At metamorphosis (stage 42, Gosner 1960), each tadpole was weighed to the nearest mg after soaking up excess water with a paper towel. Total length of four randomly chosen tadpoles (hatched) from each jar was measured in a stereoscope to the closest 0.06 mm from the jars in 15°C. Two measurements were made per female of egg diameter to the nearest 0.06 mm using a stereoscope (n = 10).

Saprolegnia culture

The pathogenic fungi *Saprolegnia spp.* was collected from a dead moor frog (*Rana arvalis*) on 21 March 2005 from a different pond to the ones used for collecting adult frogs. The fungal growth was visible to the naked eye and the fungus was identified as a

Saprolegnia species by Prof. N. Hallenberg, Department of Botany, University of Gothenburg. A small sample of fungus from the dead frog was cultured on agar (half strength Difco Emerson YpSs Agar) as per instructions by the manufacturer. A second pure culture was then topped with boiled hemp seeds and placed in 25°C for three days for standardized sampling of *Saprolegnia* (see e.g. Robinson et al. 2003 for a similar approach). To the naked eye, fungal growth was similar between the different plates and days. There was no obvious variation among hemp seeds in the amount of fungal growth. No further effort to quantify the fungus was therefore done.

Saprolegnia infection

Of the four jars per female and temperature, the eggs in two jars were infected with *Saprolegnia* and the other two were kept as controls. One *Saprolegnia*-infected or non-infected (control) hemp seed (vector) was placed on the eggs when the eggs had reached stage 13±1 (Gosner 1960). The hemp seed was placed centrally on top of the clutch of the fertilized egg mass and was in constant and direct contact with the egg jelly. The embryos/tadpoles were counted and scored for *Saprolegnia*-infection (infected or not infected). Undeveloped eggs were classified as *Saprolegnia*-infected if they showed any signs of *Saprolegnia*-growth, or otherwise as unfertilized. This classification did not allow us to discriminate between infertile eggs infected by *Saprolegnia* and viable eggs killed by early *Saprolegnia*-infection. However, we controlled for the proportion of infertile eggs using data from control jars in our statistical analysis. Fungal infection of eggs and embryos was obvious and clearly visible to the naked eye. Thus, no further quantitative or qualitative measurement of fungal infection was considered necessary.

Results and discussion

Paper I

Using artificial fertilization, we crossed common frogs from a large outbred and a small isolated population separated by 130 km to evaluate fitness consequences to the offspring from an outbreeding perspective. Offspring were raised in two temperatures (15 and 20ºC).

For females from the large population, tadpoles (hatchlings) were significantly smaller and more malformed in crosses with males from the small population, than with males from the large population. For offspring from females from the small population, no significant paternal genetic effects could be found. The difference in response to outbreeding between populations was accompanied with significant differences in the importance of maternal effects.

Although the smaller hatchling size could be explained by genetic differences between populations, it could also be a consequence of detrimental effects due to inbreeding, which is likely to be the reason for the higher incidence of malformations. Females from the large population suffered negative effects of a male genetic contribution from the small population, with a higher incidence of malformed hatchlings under outbreeding. Furthermore, eggs from the large population developed into smaller hatchlings when fertilized by males from the small population. If this was the result of parental genetic effects only, we would have expected similar effects in the reciprocal crosses in both populations. This was not the case, and the direction of paternal effects based on male size for females from the small population was even opposite to that predicted based on pure genetic differentiation between populations. Thus, from a female's perspective, outbreeding generates negative effects for the large, but not the small population, both with respect to offspring 'quality' and hatchling size. From a conservation biology perspective, translocation of frogs from healthy (outbred) populations to isolated, declining populations, to increase genetic variation (via gene flow) has been suggested (Seigel and Dodd 2002; Trenham and Marsh 2002). As indicated by the present study, such introductions could potentially lead to reduced

fitness, even if the populations are not separated by more than some hundred kilometres and maybe much less (Hitchings and Beebee 1997). We therefore suggest that care should be taken when introducing new genetic material to save threatened amphibian populations. Furthermore, using cost-effective IVF-techniques, potential fitness effects of translocations can be tested in the lab before real (and large) translocations are taking place.

Paper II

Using artificial fertilization, we crossed common frogs from a large outbred and a small isolated population separated by 130km to evaluate fitness consequences of the offspring at a later life history stage (metamorphosis) compared to paper I.

We found genetic divergence of populations separated by only 130km. Outbreeding resulted in an increase in metamorph size when eggs from the small population were fertilized with sperm from the large population. In the reciprocal cross, however, the pattern was in the opposite direction, with no significant effect of male population of origin. This is in contrast to our earlier manuscript (paper I) but at another life history stage. These results suggest a possible genetic effect of outbreeding for the small (possibly inbred) population on size at metamorphosis. These results contrast to our earlier results on outbreeding depression (paper I). However, the results are in agreement with the observation that the severity of relative in- and outbreeding depression depends on the life history stage at witch fitness is measured (Keller and Waller 2002).

Paper III

In this paper we investigated family, population and temperature variation in resistance of frog eggs to fungal infection by infecting moor frog eggs from six populations with the pathogenic fungus *Saprolegnia spp*. Infected (and control) eggs were raised to hatching in two temperatures (15 and 18ºC) and then scored as infected or not infected. Undeveloped eggs were scored as unfertilized.

There was a significant family effect on the degree of *Saprolegnia*-infection of eggs and embryos. We also found a higher incidence of infection at lower fertilization

success. Infection level also differed between temperatures. Furthermore, populations differed in level of infection, but in different temperatures, i.e. there was a significant temperature × population interaction effect.

The family effect could be due to genetic or maternal effects, although we cannot separate these explanatory factors in this study. One potential maternal effect is jelly thickness (and composition) and it is known that jelly thickness influences survival under acidification. It is also known that embryos are more sensitive to infection early in life (Robinsson et al. 2003) but the higher incidence of infection at lower fertilization success could not fully explain the effects of family, population or temperature on *Saprolegnia*-infection prevalence.

Paper IV

Building on the results from paper III, we asked if the family effect is a non-genetic, maternal or a genetic effect. Using a paternal half-sib design, we tested whether the family effect confirmed in paper III was due to maternal effects (e.g., jelly coat proteins) or genetic variation. Our results unambiguously showed that male identity can explain the degree of infection, i.e., we confirmed a paternal genetic effect on fungus resistance. Furthermore, relatively more eggs were infected when eggs were fertilized by sperm from the same, compared to a different, population. This effect was independent of variation in fertilization success. This means that there is a genetic component in embryo resistance to fungal infection in moor frog embryos, and, consequently that resistance to pathogen infection can evolve towards higher (or lower) resistance.

Paper V

In order to assess any long-term consequences of embryonic *Saprolegnia* exposure, we raised tadpoles from experiment III and IV to metamorphosis. All jars were thoroughly cleaned and disinfected before the start of this experiment and none of the tadpoles in the '*Saprolegnia*' jars showed any signs of infection. Furthermore, mortality did not differ between the jars. At metamorphosis all tadpoles were weighed to the nearest mg.

Pre-hatching exposure to *Saprolegnia* reduced the size at metamorphosis with 20 % on average. Counter to our hypothesis however, there was no difference in time to metamorphosis between treatments, suggesting that the results do not reflect an escape strategy from a compromising rearing environment (as shown in other studies e.g. Warkentin et al. 2001; Loman 1999). Although we cannot explain the mechanism behind these results, one possibility is that the immune system of tadpoles in '*Saprolegnia*' jars were stressed by the fungus and led to higher energetic demands (although they were not infected), and that subsequent growth was limited by lack of resources.

Conclusions

Many frog species show genetic divergence at relatively short geographic distances, a pattern likely to become increasingly pronounced as a result of ongoing habitat fragmentation and destruction. In such a situation, it seems that one preventive action to increase survival may be to increase gene flow by translocating frogs between populations, and in that way hinder inbreeding and loss of genetic variation. Our results indicate that care should be taken with such translocations because of the risk of outbreeding depression. Furthermore, we show that different outcomes can be expected depending on at which life history stage we estimate components of fitness. Clearly, we know too little about long-term consequences of outbreeding. Overall, among-population heterogeneity in genetic architecture makes it difficult to assess short/long time results of different population crossings.

Our work also shows that there is intraspecific variation in response to stressors among moor frog clutches (families). We show that this variation is likely to be explained by genetic differences among sires. For example, this probably explains the identified genetic component of embryo resistance to fungal infection in moor frogs. The reason for high levels of genetic variation in moor frog populations remain unknown but could possibly be a result of fluctuating exposure to fungal infection coupled with costs of tolerance under non-infected conditions. Further studies in natural populations are required to directly assess the causes and consequences of genetic variation in resistance to *Saprolegnia* for population dynamics. However, our results suggest that pre-hatching

exposure to *Saprolegnia* reduces the size at, but not the time to, metamorphosis for moor frog tadpoles. Because size at metamorphosis is an important fitness trait in frogs with additional demographic consequences (e.g., Altwegg and Reyer 2003), these results suggests that fungal exposure may have carry-over effects on populations that are not predictable on the basis of embryonic mortality.

Svensk sammanfattning

Med hjälp av artificiell befruktningsteknik korsade jag grodor från olika populationer för att utvärdera konsekvenser hos avkomman ur ett utavelsperspektiv, och för att utvärdera kvantitativa genetiska effekter på immunologisk resistans mot en skadlig svamp (*Saprolegnia*). I de två första artiklarna korsade vi vanlig groda (*Rana temporaria*) från två olika stora populationer. Vi fann att honor från en stor population led av effekter av hanens genetiska bidrag om han kom från en liten population, med högre andel missbildade grodyngel vid utavel. Från en honas perspektiv gav utavel negativa effekter för den stora, men inte den lilla populationen, båda med avseende på avkommans kvalitet och grodynglens storlek. Vår studie visar att translokationer (förflyttningar av djur mellan populationer) kan leda till minskad fitness även om populationerna inte är separerade med mer än några hundra kilometer och kanske mycket mindre. Vid metamorfos led utavel till en ökning av metamorfosstorlek när ägg från den lilla populationen blev befruktade av spermier från den stora populationen. Detta står i kontrast mot resultaten från vår tidigare studie (ovan) på ett annat livshistoriestadium. Dessa resultat pekar på en möjlig genetisk effekt av utavel på storlek vid metamorfos i den lilla populationen.

Experimentell infektion av åkergrodeägg (*Rana arvalis*) med den patogena svampen *Saprolegnia* visade en tydlig familjeeffekt på graden av ägg- och embryoinfektion, speciellt vid låg befruktningsgrad. De flesta familjer var mer infekterade vid lägre temperatur. Vi fann en genetisk effekt från fadern på resistans mot svampinfektion. Dessutom blev fler ägg infekterade när äggen befruktades av spermier från hanar från samma, jämfört med en annan, population.

Känsligheten för svampinfektioner är komplex och utbrott av svampangrepp är svåra att förutsäga. Vi fann att grodyngel som före kläckning hade varit utsatta för *Saprolegnia* metamorfoserade vid mindre storlek. En tänkbar förklaring skulle kunna vara att immunsystemet hos grodyngel utsatta för *Saprolegnia* blev stressade av svampen och att detta ledde till högre resursförbrukning (trots att de inte själva fick en infektion), och att tillväxten sedan blev begränsad av resurstillgång. Detta vore överraskande eftersom ett flyktbeteende från en ”dålig” utvecklingsmiljö (visat i andra studier) brukar vara att metamorfosera tidigare och vid lägre vikt (för att på så sätt fly från t.ex.

predatorer eller uttorkning). Sammanfattningsvis så visar våra resultat att grodpopulationer är genetiskt separerade även mellan relativt små geografiska avstånd och att konsekvenser av in- och utavel på respons mot stressorer är svåra att förutsäga.

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