Review

Global Amphibian Declines, Loss of Genetic Diversity and Fitness: A Review

Morten E. Allentoft [1],* and John O'Brien [2]

[1] School of Biological Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

[2] Brannoxtown, Trim, Co. Meath, Ireland; E-Mail: johnob@ucd.ie

* Author to whom correspondence should be addressed; E-Mail: mea39@student.canterbury.ac.nz; Tel.: +61-422502486.

Abstract: It is well established that a decrease in genetic variation can lead to reduced fitness and lack of adaptability to a changing environment. Amphibians are declining on a global scale, and we present a four-point argument as to why this taxonomic group seems especially prone to such genetic processes. We elaborate on the extent of recent fragmentation of amphibian gene pools and we propose the term *dissociated populations* to describe the residual population structure. To put their well-documented loss of genetic diversity into context, we provide an overview of 34 studies (covering 17 amphibian species) that address a link between genetic variation and >20 different fitness traits in amphibians. Although not all results are unequivocal, clear genetic-fitness-correlations (GFCs) are documented in the majority of the published investigations. In light of the threats faced by amphibians, it is of particular concern that the negative effects of various pollutants, pathogens and increased UV-B radiation are magnified in individuals with little genetic variability. Indeed, ongoing loss of genetic variation might be an important underlying factor in global amphibian declines.

1. Introduction

Biodiversity is in the midst of another period of mass extinction, probably comparable to those of the palaeontological past [1,2]. However, the fundamental difference with regard to the current crisis is that it has not been brought about by stochastic or catastrophic events, but is instead directly attributable to anthropogenic effects (e.g., [3,4]). With more than 40% of the World's amphibian species in decline [5] and an estimated extinction rate over 200 times that of their natural background rate [6], amphibians are possibly of greater conservation concern than any other vertebrate group (although see [7]).

The proposed factors behind these declines are almost as many as they are complex. Amphibians are generally believed to be sensitive to environmental perturbations, partly because of their central place in the food chain (being both prey and predator), because they often utilise both terrestrial and aquatic habitats, and can have very different feeding ecologies at different stages of their life cycles. Recent research has documented and suggested an array of causes associated with this crisis. In addition to the ongoing and very obvious destruction and fragmentation of habitat [8-11], these include effects of climate change [12,13], increased UV-radiation due to ozone depletion (e.g., [14,15]), predation or competition by invasive species [16,17], pollution (e.g., [18,19]), road-kill [20,21], over-harvesting for human consumption [22] and diseases [23,24]. Of particular concern is the rapid and global spread of chytridiomycosis (e.g., [25,26]), caused by a pathogenic fungus, which has been described as posing the greatest threat to biodiversity of any known disease [1]. Further, many of these factors can work synergistically in complex interactions [27,28]. For example, it has been argued that climate change has caused an increase in virulence of the chytrid fungus [29]. Similarly, increased UV-penetrance [30] and agro-chemicals [31] have both been shown to cause immuno-suppression in amphibians, thereby further increasing their vulnerability to circulating pathogens.

In this review we deal with another factor which, perhaps, has been somewhat overlooked in discussions of this topic, *i.e.*, genetic effects as a potential key factor in amphibian global declines and, in particular, reduced fitness due to eroded genetic diversity. We summarise the available literature to investigate the extent to which lower genetic variation renders amphibian populations prone to reduced fitness. It seems very timely to provide an overview of our current knowledge on this subject, because identifying the key factors contributing to the declines is more important than ever for these highly endangered animals and, although the issue of low genetic variation has been addressed in many isolated studies of amphibians, no attempt has been made to compile this information in a cohesive and comprehensive review. It is also timely because, while maintaining a high level of genetic diversity is ever so often hailed as an important objective in many conservation contexts, its critical importance in wild populations remains controversial and more clarification is needed to understand the underlying processes and their relative importance.

The concept that populations with low levels of genetic variability possess higher extinction risk is not new [32,33]. Populations with low genetic variation have a higher probability of becoming genetically inbred (see Textbox), with the potential consequence of lowered fitness. Furthermore, the inherent genetic variability of many populations is considered 'adaptive' to changing environmental conditions, thereby acting as a buffer against stochastic and catastrophic events (e.g., [34,35]). For that reason, genetic diversity is perceived as a vital pillar of biodiversity, deserving of protection under

international conventions and national legislature, such as the Convention on Biological Diversity (CBD 1992), the Habitats Directive of the European Union (Council Directive 92/43/EEC 1992), and the National Strategy for the Conservation of Australia's Biological Diversity (DEST 1996), amongst others.

1.1. Why Are Amphibians Particularly Vulnerable

Our objective here is not to present a treatise on the *theory* behind genetic problems in small populations (but see Textbox for an overview) as detailed discussions of this topic have been presented elsewhere for a vast array of fauna. Here we focus specifically on amphibians, but before engaging in a review of the literature examining genetic diversity-fitness correlations (GFCs), we need to clarify why the discussion is particularly relevant to this taxonomic group. Below, we present a four-point argument as to why we believe amphibians are prone to severe loss of genetic diversity in recent times. They include a combination of obvious anthropogenic impacts and natural life-history traits that operate synergistically to increase the impact of genetic effects: (1) amphibian breeding strategy; (2) recent, vast and rapid declines in population sizes; (3) habitat fragmentation; (4) a typically low dispersal capability compared to most other vertebrates.

1.1.1. Small effective population sizes and whole clutch mortality

Loss of genetic diversity by genetic drift and inbreeding is directly linked to the effective population size (see Textbox). In large stable populations with random mating and many breeders each year, genetic drift and inbreeding are minimal and genetic diversity is maintained across generations. Amphibians often have very low effective population sizes [36,37], with just a small proportion of the reproductively-capable individuals contributing to the gene pool each mating season [38-40]. Also, breeding success and mortality rates can be highly variable between years [41-43]. While overlapping generations, or "genetic compensation" through a decrease in polygonous matings [44], may provide a buffer in this context and minimise unfortunate genetic effects, a few consecutive "bad" years, can cause a highly fluctuating population size with strong genetic drift as a consequence [45]. Equivalent to a very low number of effective breeders, genetic drift can reach extreme levels if survival among the offspring is genetically non-random. For example, if only one or a few "lucky" egg clutches survive predation or a desiccation event, an entire cohort can, in theory, be comprised solely of siblings. This issue of selective mortality causing fast genetic drift has been discussed mainly in relation to directly-developing amphibians, where extreme levels of genetic differentiation have been observed among populations even in continuous habitat (e.g., [46,47]). Dubois [48] takes this theory one step further, and suggests that dramatic genetic drift events by whole clutch mortality could perhaps explain the tremendous and fast radiation of amphibian fauna on Sri Lanka.

1.1.2. Population declines

This point is arguably the most straightforward and needs only brief mention here. Over 40% of known amphibian species are considered to be in decline [5,49] and therefore many local amphibian populations must be experiencing a depletion of genetic diversity due to increased genetic drift and inbreeding (see Textbox). Destruction of habitat, introduction of competitors or predators, road-kill, pollution and other environmental hazards compound the effects of already small effective population sizes and thus, reinforce the negative genetic effects attributable to small populations. A direct link between genetic variability and population size in wild populations was documented in a large, influential meta-study by Frankham [50], and this association has also been shown specifically for amphibians [51,52]. In terms of maintaining genetic diversity, small effective population sizes and population declines do not necessarily impose a problem if a genetic influx is maintained to counteract the effects of drift in the local gene pools. However, as we discuss below, gene flow is effectively prevented in many localities today.

1.1.3. Habitat fragmentation

This point is central to our discussion and, therefore, we discuss it here in some detail. Many amphibian species in natural environments can be considered to form meta-populations [43,53] due to their requirements for non-continuous habitat types (e.g., ponds). The gene flow and colonisation dynamics that characterise meta-population systems ensures that equilibrium between founding and extinction of localised populations is maintained (e.g., [54]). It is widely acknowledged that anthropogenic fragmentation of natural habitats constitutes one of the greatest threats to terrestrial biodiversity [55-58], but also from a strictly genetic viewpoint difficulties can arise when large contiguous populations become fragmented, or former meta-populations transform into isolated islands of sundered populations. As a result, the isolated sub-populations become the units on which genetic drift, inbreeding and selection act [35,59-61] and without the ameliorating influence of gene flow, their concerted effects impose a more rapid erosion of genetic diversity, exacerbating fitness reductions and extinction risks. A large number of studies have documented the negative impacts of recent habitat fragmentation on amphibians (e.g., [8,9,62,63]) but, in relation to the present review, the most illustrative ones are those comparing genetic variation and/or gene flow in fragmented *versus* non-fragmented landscapes in populations of the same species. For example, Arens *et al.* [64] compared genetic diversity in moor frog (*Rana arvalis*) populations in two areas fragmented by roads and agriculture, but differing in time since the establishment and intensity of the barriers. Higher genetic differentiation and lower genetic diversity was documented among sub-populations in the most intensively cultivated area. Andersen *et al.* [51] provided evidence of how fragmentation has contributed to bottlenecking and subsequent inbreeding in the European tree frog *Hyla arborea*. Hitchings and Beebee [65] showed lower genetic diversity and twice the differentiation among urban common frog (*Rana temporaria*) populations compared to populations from rural habitat, despite study sites in the urban setting being in greater proximity. Lesbarrères *et al.* [66] documented profound genetic structuring and significantly lower genetic variation in sub-populations of agile frog (*Rana dalmatina*) sampled on either side of a highway, compared to populations sampled far from trafficked

roads. Large roads have also been identified as effective barriers for common frog (*Rana temporaria*) dispersal [67] and, similarly, Vos *et al*. [68] showed that roads and railways worked as barriers to gene flow and represented a higher explanatory value to the genetic differentiation between populations of moor frog (*Rana arvalis*) than geographic distance between populations. A study by Spear and Storfer [69] emphasized that fragmentation can take many shapes, e.g., tailed frogs (*Ascaphus truei*) prefer low levels of solar radiation, explaining why absence of forest (whether due to natural changes or anthropogenic activity) was identified as a key restrictor to gene flow. Likewise, recent logging accounted for higher genetic differentiation and lower genetic diversity in populations of giant salamander (*Dicamptodon tenebrosus*) [70]. Although associations of recent habitat fragmentation and genetic variation in amphibian gene pools have been most intensively examined in Europe and North America, the trend is by no means unique to the northern hemisphere (e.g., [71-74]).

Low genetic diversity in amphibian populations is of course not always attributable to human activities. For example, a clear valley-mountain effect has been demonstrated in Columbia spotted frogs (*Rana luteiventris*), with a negative correlation of genetic diversity and elevation [75]. The agile frog (*Rana latastei*) has demonstrated a strong east-west gradient in genetic diversity, suggesting a loss of genetic variability through numerous consecutive founder effects as the species expanded westward from a glacial refugium in Eastern Europe [76]. Similarly, a study of the Natterjack toad (*Bufo calamita*) across Europe revealed a remarkable decrease in genetic diversity with increasing northern latitude that could not be easily attributed to anthropogenic effects [77]. In an elegant study, Ficetola *et al*. [78] were able to show that the genetic signal in *Rana latastei* was jointly shaped by postglacial colonization patterns and recent fragmentation, but that fitness, in this case hatching success, was only affected by the latter. It can be a difficult task to discriminate between low genetic diversity as a result of recent fragmentation or as a remnant of the phylogeographic history (e.g., [62]), but from a conservation perspective, an attempt to make the distinction is important. The risk of inbreeding depression is greatly enhanced in the former; whereas populations with a long history of low diversity may have had time to purge deleterious alleles by natural selection (see Textbox).

We propose the term *dissociated populations* to describe the residual populations that arise where former meta-population structures have experienced major disruption to gene flow because of human-mediated loss of connectivity in the landscape. As discussed above, this has serious negative implications for the preservation of genetic diversity in many local amphibian gene pools.

1.1.4. Low dispersal rates

Amphibians are often described as highly site-philopatric and demonstrate poor dispersal capabilities, even in natural environments [79-83]. This is commonly explained by a dependence on moist habitats and slow terrestrial movement capabilities [84,85]. Although these limitations are clearly not true for all amphibians and should not be accepted by default [86,87], it is not unreasonable to use this generalisation when comparing amphibian migration rates [88] or levels of inter-population genetic differentiation (e.g., [47,89]), with that of other vertebrates. Consequently, many amphibian sub-populations might be prone to high, background genetic drift because of limited connectivity, causing a state of natural fragmentation of the gene pool. Further, in modern landscapes, genetic influx is often severely reduced, making it even more difficult for dissociated populations to counter-balance

the effects of genetic drift on local gene pools. Low recruitment of dispersing individuals is believed to play a major role in amphibian declines in fragmented landscapes [9,90,91] and the genetic effects of restricted gene flow are, as discussed above, well-documented. From a long-term genetic viewpoint, species of low vagility seem more vulnerable to anthropogenic obstructions since even moderate fragmentation can completely hamper gene flow. However, it has been argued that highly dispersing amphibian species actually suffer more from fragmentation in the short term [9]. This seems somewhat counter-intuitive, but dispersing amphibians are exposed to higher mortality risk in modern landscapes, for example as road-kill [92]. Likewise, Funk *et al*. [93] suggested that the introduction of barriers in the landscape has more severe and immediate effects on species with high dispersal rates, since this trait plays an important role in their overall population survival, compared to species adapted to a less dynamic situation.

While we have no intention of presenting a single stereotyped image of amphibians, we still argue that a combination of the four points mentioned above apply to the vast majority of amphibian populations. Consequently, it seems reasonable to suggest that the current loss of genetic diversity in amphibians is likely to be greater than in many other taxa (especially vertebrates). However, large scale meta-studies, beyond the scope of this article, are needed to examine that hypothesis.

2. Genetic Diversity and Fitness in Amphibians

In the previous section we have outlined why we believe amphibians are experiencing a rapid and ongoing loss of genetic diversity in many parts of the world. Here, by reviewing the available literature, we assess the impact of this "molecular erosion" on fitness in amphibians. We discuss the findings of 34 published studies with direct relevance to this theme (see Appendix 1). The considerable number of publications addressing what might appear to be a narrow, taxon-specific topic serves to confirm our contention of a general concern shared by many researchers in the field, and also the relevance of compiling and interpreting the available information in this review.

2.1. Genetic-Fitness Correlations

Firstly, how and why do genetic-fitness correlations (GFCs) arise? Finite populations lose genetic variability due to genetic drift, and a distinct negative correlation is expected between loss of genetic variation and individual/population fitness (see Textbox). In support of this, reduced fitness (*i.e.*, inbreeding depression) has been reported from practically all species where inbreeding has been documented [94,95]. Though many publications show that GFCs exist and can sometimes be established with surprisingly limited genetic information compared to optimal theoretical predictions, the underlying mechanism is still debated. Three hypotheses have been proposed for a positive association between genetic variability and fitness (reviewed in [96]): (1) a 'direct effect' whereby GFCs arise through selection acting directly on the loci under study, with higher fitness in heterozygotes (overdominance). This is pertinent to the study of, for example, the MHC genes involved in immuno-competence; (2) a 'local effect' where selection acts on genes linked to the molecular markers being studied and GFCs arise because of linkage disequilibrium (associative overdominance) and (3) A 'general effect' where genetic variation at the molecular markers under

study reflects the level of genome-wide heterozygosity, and variance in fitness is caused by variance in inbreeding. Obviously, the 'direct effect' hypothesis can only be applicable to molecular markers subjected to selective pressure, such as allozymes. Microsatellites, on the other hand, are neutral genetic markers and the most widely used in present day population genetics. Though GFCs have been established countless times with these markers, the degree to which microsatellites represent genome-wide heterozygosity has been questioned [97], and the relative importance of local and general effects remains controversial (e.g., [98]). Allozyme data, often used in earlier GFC studies, typically have low variability and hence little power to detect true differences in genome-wide heterozygosities between individuals. While highly variable microsatellites may be preferable due to their selective neutrality, they lack the potential 'direct effect' offered by allozymes. Moreover, Lesbarrères *et al*. [99] showed how detectability of GFCs was highly environmentally-dependent in *Rana temporaria* populations. Thus, GFC studies should preferably be carried out in a context-driven setting. This point is perhaps corroborated by quick review of Appendix 1 where, in those two instances where tests were made concurrently in wild and laboratory set-ups, only the wild populations demonstrated significant GFCs. Despite these caveats, meta-analyses by Reed & Frankham [100], Coltman & Slate [101] and Chapman *et al*. [102] generally found weak but yet positive and significant correlations between proxies of genetic variability and fitness from a variety of datasets representing many taxonomic groups.

2.2. Review of GFCs in Amphibians

The 34 publications that we consider here represent 17 species of amphibians: 16 anurans and one caudate (Appendix 1). Published works dealing with GFCs in caecilians could not be found; although Gower and Wilkinson [103] reviewed the conservation status of this enigmatic amphibian group. The bias towards the anuran Order may reflect species richness within the group, but also the relative ease with which large numbers of anuran eggs and larvae can be collected and reared in a controlled environment. We have not attempted to carry out quantitative meta-analyses on these datasets, as we feel the experimental set-ups are so heterogeneous as to render any potential correlations meaningless and because comprehensive investigations of GFCs already exist [100-102]. This review simply serves to provide an overview to examine the extent and context in which these genetic effects have been identified in amphibians. Nineteen of the 34 publications we consider directly investigated a correlation between a measure of genetic diversity and fitness (Appendix 1). In the remaining 15 studies, the authors based "GFC-like" interpretations on more indirect evidence from, for example, the experimental setup (e.g., [104]), the known demographic history of the sampled populations [90,105], or by correlating fitness with other relevant genetic measures such as the inbreeding coefficient [51] or degree of genetic isolation [65], rather than genetic variability *per se.* For every fitness trait investigated in each study, we have listed a simple "yes" or "no", respectively confirming or rejecting a GFC or a GFC-like association (Appendix 1).

Perhaps displaying the omnipresence of the problem and/or a relative ease of testing these associations in amphibians, GFCs have clearly been documented in many amphibian species and for many different fitness traits. Likewise, strong correlations have been described using different types of genetic markers, for both wild populations and laboratory set-ups, and under normal as well as

stressful conditions (Appendix 1). In fact, for 15 of the 19 publications directly investigating GFCs, a positive correlation was demonstrated in one or more of the fitness traits under scrutiny suggesting that the individuals were affected in at least one life stage. In nine of these studies, GFCs were documented in all investigated fitness traits. In contrast, only four of these 19 studies rejected an association in all measured fitness traits (Appendix 1). So, there is a considerable body of evidence in favour of GFCs in amphibians, albeit offset by a number of studies where such links were not uncovered (as discussed further below). In addition, convincing GFC-like associations have been shown in several studies (Appendix 1). However, a qualitative assessment of the documented GFCs in amphibians seems more appropriate here than actual numbers, which are likely skewed by a publication bias [102]. Also, it is important to note that an individual needs only to be affected in one trait to be affected overall.

2.2.1. Measures of fitness

In the first publication to assess a link between fitness and genetic diversity in amphibians, Nace *et al.* [104] showed in 1970 how post-metamorphic survival considerably declined in highly inbred lineages of *Rana pipiens* in the laboratory. The objective of that particular study was not related to understanding the ecology of amphibians, but rather, the observation of lowered fitness was simply used to confirm the successful development of gynogenetic individuals, which are useful in experimental genetic research. Samollow and Soule [106] and Pierce and Mitton [107] represent the first attempts to directly correlate genetic diversity (measured as heterozygosity in allozyme loci) with fitness traits in amphibians, and here the results are indeed discussed in an ecological context. Both of these studies demonstrated how survival and larval growth in wild populations are clearly linked to levels of genetic diversity and they successfully managed to assess the importance of genetic diversity for survival during different life history phases. For example, while the periods of winter and metamorphosis are both highly stressful for amphibians, causing increased mortality, only survival through the former phase appears to be correlated with heterozygosity [106]. The authors hypothesise that the genetic basis for surviving climatic events that vary in time, space and severity must be very different from that underlying survival under a developmental constant such as metamorphosis.

Of the >20 different fitness-related traits listed in Appendix 1, **survival** (under a variety of settings and life stages) is arguably the most prominent fitness parameter and certainly the most frequently assessed. This is, conceivably, because survival is the fitness attribute most exposed to selective pressure, and therefore most likely to reveal a correlation where one exists. The most illustrative example of survival being directly linked to genetic diversity is provided by Schmeller *et al.* [108], who compared the age of individual adult *Rana perezi* with allozyme variability and showed a significant positive correlation. Their conclusion was that the more genetic variation a frog possesses, the longer it lives. Hitchings and Beebee [109] showed a significant relationship between larval survival and genetic diversity in *Bufo bufo* populations and, in accordance with our discussion above of habitat loss and genetic effects, isolated urban populations appeared much less viable than their rural counterparts. A very similar pattern was apparent in *Rana temporaria* populations in the study by Johansson *et al.* [110], where small isolated populations displayed less genetic variability and higher larval mortality than populations in continuous habitat. Thus, again, direct links emerge between recent, human-mediated changes of the landscape and genetic diversity and fitness. In addition, that

same study also demonstrated a significant loss of variation in quantitative genetic traits in the fragmented landscape, suggesting that these amphibian populations are not only experiencing reduced fitness but also a serious loss of adaptive potential. The authors concluded: "...our results indicate that agriculturally-induced habitat fragmentation may increase the role of random genetic drift to such an extent that both genetic variability in neutral marker genes and mean values of fitness-related traits are reduced..." Likewise, Halverson *et al*. [111] documented a staggering seven-fold increase in survival from the least to most heterozygous individuals within a single *Rana sylvatica* population, measured in the wild.

Growth, and especially larval growth, has been assessed in many of the publications listed in Appendix 1. Large larvae often have lower predation risk, are competitively superior to smaller larvae, and metamorphose earlier and at larger body size, which is closely linked to post-metamorphic fitness (summarized in [112]). However, this fitness parameter appears less intimately linked with genetic diversity measures than survival. Eight studies document a GFC or GFC-like association using larvae growth, whereas another five find no such association. This is perhaps not surprising, since impaired growth is a less detrimental factor than survival in terms of overall fitness, and hence the selection pressures operating on this trait must be less pronounced. Regardless, the results of several studies confirm a direct relationship between growth and genetic diversity (e.g., [99,107,110,113]). Interestingly, Rowe *et al*. [52] detected a significant GFC in growth rates of *Bufo calamita* larvae and their variability at eight microsatellites, but no correlation with larval survival, demonstrating that the relative strength of these signals is highly variable and can only truly be assessed on a case-by-case basis.

The propensity for **physical abnormality** in larvae also represents a fitness trait often assessed in amphibians (Appendix 1). In an allozyme survey of British *Bufo bufo* populations, Hitchings and Beebee [109] documented a correlation between low genetic diversity and physical abnormalities in small isolated populations found in urban environments. Similar effects have been observed in *Rana sylvatica* [15] and *Rana temporaria* [65], although the failure to document any correlation between malformations and individual heterozygosity within a population of *Ambystoma tigrinum* [114] confirms that other factors are also responsible for amphibian deformity. Indeed, physical abnormalities may arise due to a variety of factors, and investigating GFCs in this context without considering other biotic and abiotic factors, could prove misleading.

Mitton *et al*. [115], measured **oxygen consumption** as a proxy for fitness in *Ambystoma tigrinum* and correlated it with genetic variation in allozymes. Interestingly, active O_2 consumption (the aerobic capacity) of an individual was positively correlated with its genetic variation; whereas standard O_2 uptake (cost of maintenance) showed a negative correlation. Both results suggest a very direct fitness advantage in heterozygotes. The study of Rowe and Beebee [116] on *Bufo calamita* is notable in that the experimental set-up facilitated investigation of **competitive potential** in two populations that differed markedly in levels of genetic variation and inbreeding. Individuals from the large genetically diverse population performed significantly better in competition, even when reared in a shared pond representing the local environment of the small, inbred population. In this context, any adaptations conferring local advantage on the small inbred population appear to be largely over-ruled by much greater general fitness among individuals from the genetically diverse population. **Clutch size** [117] and **hatching success** [78] have both shown a significant GFC. The latter study is especially

interesting in that it effectively manages to isolate the effects of long term genetic loss due to long term dispersal patterns from more recent habitat fragmentation, with fitness only negatively affected by the recent genetic loss, again providing a compelling argument for the direct link between human-mediated landscape changes and reduced amphibian viability because of genetic effects.

2.2.2. Limitations

Not all of the studies listed in Appendix 1 could detect a genetic effect on fitness. Several explanations for this are available, but the most obvious one is simply that no GFC exists. If only limited variance exists in the level of inbreeding among individuals, for example because the entire population is either highly inbred or highly outbred, then no correlation is expected [98,118]. This could perhaps explain why no GFCs were detected in studies of large healthy populations of *A. tigrinum* [114], *B. calamita* and *R. temporaria* [119] in contrast to studies that compared individuals from relatively inbred and outbred populations (e.g., 51,116]). On the other hand, the latter approach has been criticised because environmental heterogeneity over the geographic range sampled can potentially create spurious relationships [120]. Other reasons for an apparent lack of GFCs could be small sample sizes or limitations in the genetic markers applied. Chapman *et al*. [102] emphasize that these correlations are normally weak and that many highly polymorphic microsatellites are needed to effectively detect them. The limitations in less variable markers such as allozymes would be even more pronounced, unless loci under direct effects (displaying overdominance) are used. Also, the investigated fitness trait(s) might not be under great selection pressure in the given experimental setup. In general, life history fitness traits are believed to be more strongly affected by inbreeding compared to morphological and physiological traits [101]. This prediction is perhaps reflected in Appendix 1 where survival—a life history trait—appears more intimately linked with genetic variability than growth, which is a morphological trait. Finally, purging of the mutational load (see Textbox) in previous generations might have obscured an otherwise expected relationship between heterozygosity and fitness. Despite these limitations, the fact that associations between genetic variability and fitness are documented so vigorously here confirms the applicability of these approaches and, more importantly, provides a convincing argument for the contribution of genetic factors to fitness components and the overall viability of amphibian populations. Although representing a highly interesting topic, it is slightly less important, from a conservation perspective, whether these associations arise through direct, local or general effects.

2.2.3. GFCs and synergisms

We now turn our focus towards a few very pertinent studies that are of crucial importance to understanding the magnitude of some of the threats amphibians presently face (summarized in our Introduction). It is well known that low levels of genetic diversity can be accompanied by higher susceptibility to emerging pathogens [121-124]. Thus, it is particularly worrying that one of the greatest direct threats to amphibians appears to be from pathogens. Different strains of Ranavirus, for example, are responsible for amphibian mass mortality in Europe and the USA [125]. Pearman and Garner [126] tested susceptibility to this virus in individuals from populations with low *versus* high

genetic diversity in *Rana latastei*. As suspected, limited mortality was recorded in individuals from the genetically variable population, whereas there were few survivors in the genetically depauperate population. As mentioned in the Introduction, the global occurrence of the pathogenic chytrid fungus is of major concern in amphibian conservation, but despite an urgent relevance, we could not find any study that examines a direct link between susceptibility to chytridiomycosis and genetic variability in the host. However, Parris [127] investigated the response to chytrid infection in artificial hybrids compared to pure strains of two closely-related ranids and documented a reduction in several fitness traits in all strains, but greater effects were seen among hybrids. This study suggests that the fungus attacks a variety of genotypes indiscriminately, but also that severe outbreeding can have a negative effect on individual resistance to the disease. The latter result is perhaps not surprising, since other negative outbreeding effects, in terms of larval malformations and size, have been documented even within the same ranid species in crosses between two populations separated by just 130 km [89].

Bridges and Semlitsch [128] showed that variation in the ability of *Rana sphenocephala* (*Lithobates sphenocephalus*) larvae to tolerate an insecticide was closely linked with additive (heritable) genetic variance, indicating that resistance to such pollutants could perhaps evolve, but only if a high level of genetic diversity was maintained. Pierce *et al.* [129] uncovered significant differences between survival of larvae from different egg clutches when exposed to low pH, and hypothesised an additive genetic basis for this effect. Several other studies have documented a clear genetic basis for local adaptations in fitness-related characters in amphibians investigated under a range of set-ups [130-133]. Such results emphasize the crucial evolutionary importance of maintaining high levels of genetic diversity, especially where amphibians are challenged with the introduction of novel chemical compounds and physical alterations of the environment, which can require a rapid adaptive response to prevent extinction.

Increased exposure to UV-B light due to recent ozone depletion is believed to be one of many important factors in amphibian declines (e.g., [134-136]). Weyrauch and Grubb [15] examined the synergistic effect of reduced genetic diversity and UV-B exposure and showed that populations of *Rana sylvatica* with low genetic diversity had higher larval mortality rates than populations with higher diversity when both were exposed to direct sunlight. Because of the well-documented and on-going genetic erosion of amphibian gene pools, these synergistic effects between genetic diversity and known threats are a major concern and emphasise the difficulties in isolating cause-and-effect relationships if simplified tools and single parameters are employed.

These latter studies provide a tantalizing glimpse as to how GFCs may underlie global amphibian declines. While a variety of causes have been suggested and, indeed, demonstrated in recent years, the effects of these threats are propagated by genetic effects and their concomitant contribution to individual and/or population fitness. We have only considered briefly the role of additive genetic variation and heritabilities in this review, although they undoubtedly contribute to the ability of amphibians to respond to novel threats given their link to evolutionary potential.

3. Conclusions

A growing body of research shows that many amphibian populations are experiencing a depletion of genetic variation and we offer a four-point argument to explain this progressive deterioration in

genetic variability. We introduce the term *dissociated populations* to describe the effect of contemporary loss of habitat connectivity, causing fragmented gene pools and allowing genetic drift to operate on small isolated units. The link between modern anthropogenic landscape modifications and loss of genetic diversity is direct, unambiguous and well-documented.

Theory predicts that loss of genetic variation will lead to reduced fitness because of inbreeding effects and loss of adaptability and, indeed, in the vast majority of the 34 publications assessing this link in amphibians, clear correlations have been presented. We note that GFCs have been demonstrated across many different fitness traits in amphibians, using a variety of genetic markers and are evident both in laboratory settings, as well as wild populations. Therefore, it is hardly controversial to suggest that the ongoing reduction of genetic diversity is significantly reducing fitness in many wild amphibian populations. Controversy may arise, however, in trying to establish the relative importance of genetic factors compared to more direct threats, such as habitat destruction or spread of chytridiomycosis. General discussions on the role of genetic effects in extinction have been provided elsewhere, with examples both in favour of (e.g., [137,138]) and against (e.g., [139]) the process.

This review is not aimed at resolving that particular dispute, but we do wish to emphasize that an assessment of isolated effects of low genetic diversity (e.g., inbreeding depression) can rarely provide a complete picture of the potential magnitude of the problems facing threatened species. Several of the papers included in Appendix 1, show that GFCs are often more pronounced and negative genetic effects more severe under stressful conditions. Hence, concluding that populations without overt signs of inbreeding depression are genetically viable, might prove short-sighted. Perhaps even more importantly, this review also highlights that the effects of several currently know threats to amphibians are magnified when low genetic variability prevails. Therefore, assessment of the impacts of these threats would likely gain accuracy and credibility by also discussing their genetic element. Although large-scale meta-studies are imperative to investigate this further, we propose that genetic depletion could be a major underlying factor in global amphibian declines, increasing susceptibility to many of the direct threats amphibians are currently subjected to.

Additionally, given that the greatest (amphibian) species richness and extinction threat lies in tropical regions, efforts should be made to balance the present significant temperate species bias in studies of genetic diversity. It is extremely important to increase our knowledge on the genetic consequences accompanying the ongoing habitat destruction which takes place in many tropical areas today.

Finally, we hope that this review will serve as yet another strong argument for conservation biologists as to why population connectivity is of crucial importance in maintaining population fitness and, just as importantly, in maintaining adaptive potential in a world experiencing massive environmental and climatic changes.

4. Textbox

4.1. Genetic Drift and Inbreeding—Big 'Players' in Small Population Genetics

Each new generation of any sexually-reproducing species represents a sample of the parental gene pool. Since only one of the two (in diploids) alleles from each parent is passed on to the next

generation and not all individuals may successfully mate, or may differ in the number of progeny produced, the allele frequencies in any given locus are prone to stochastic changes across generations. In time, some alleles will either become fixed or lost to a population unless new mutations or incoming gene flow counteract these processes. This random loss of heterozygosity is termed **genetic drift** and the magnitude is inversely proportional to the effective population size (N_e). In finite populations, genetic drift will eliminate heterozygosity at a rate of $1/(2N_e)$ per generation, if no selectional constraints interfere. Thus, small populations lose genetic variation faster than larger ones.

Consider the following equations:

$$T_{fix} = [-4N_e(1-p)\ln(1-p)]/p$$

and:

$$T_{loss} = [-4N_e p \times \ln(p)]/1-p$$

where T_{fix} and T_{loss} are the number of generations expected to pass before allele fixation [140] and allele loss [141] occur, respectively. Clearly, allelic losses and fixations will occur much more rapidly in small populations. Genetic drift serves to decrease genetic variation within populations but, concurrently, increases genetic differentiation among populations because stochastic processes fix different alleles in different gene pools (e.g., [142]). Isolated populations will drift apart genetically over time, at a rate determined by the sizes of the populations.

   Inbreeding is classically considered to arise through consanguinous mating of related individuals, causing allele frequencies to deviate from Hardy-Weinberg proportions. However, in most wild populations, inbreeding effects associated with random mating (*i.e.*, genetic drift) are often more relevant. In small populations, there is a high probability that two related individuals will mate by chance and produce inbred progeny. Further, in a small population with low genetic diversity, the probability is relatively high that an individual will inherit two copies of the same allele for any given locus, thereby becoming **autozygous** for that locus. If that allele happens to be a recessive deleterious one, signs of inbreeding depression will emerge, even if the parents were essentially unrelated. The level of autozygosity in an individual describes the proportion of alleles being identical by descent (e.g., [143]), and the concomitant negative effects are indistinguishable from "true" inbreeding events between related individuals. Given that genetic drift and inbreeding in small populations are inextricably linked, we consider them together here, and their co-dependence and effects can be visualised through the mathematic relationship:

$$H_t/H_0 = [1 - (1/2N_e)]^t = 1 - F_t \text{ [140]}$$

where H_t and H_0 are measures of heterozygosity at times t and 0 respectively, N_e is the effective population size and F is the inbreeding coefficient. We can recognize the element $1/2N_e$ from above as the loss of heterozygosity due to genetic drift. Genetic drift and inbreeding events affect the gene pool by increasing homozygosity and, as a consequence, reveal the phenotypic effects of deleterious recessive alleles normally present in low frequencies. **Inbreeding depression** is manifested by a reduction in 'fitness' attributes such as survival, growth, reproductive success, *etc*. While the impact of increasing homozygosity of recessive *lethal* alleles on 'fitness' is obvious, it is often the effect of sub-lethal alleles that contributes most to inbreeding depression and extinction risk [144]. Recessive lethal alleles are exposed to strong selection pressure and thus are rapidly eliminated (purged) from the gene pool [145]. In contrast, sub-lethal alleles can persist, both as homozygotes and heterozygotes, and

can even increase in frequency in small populations where strong genetic drift might override the purging effects of selection. In any case, the efficiency of purging has been questioned recently [146] even if such alleles could be purged, new sub-lethal alleles will arise by chance through mutation and as a result, a **mutation-selection balance** persists. In addition, **balancing selection**, either associated with heterogeneous environmental conditions [147] or through **overdominance**, plays a significant role in the maintenance of genetic diversity at 'fitness' loci. For example, there are instances where heterozygous individuals at some loci are 'fitter' (overdominance) than homozygotes, even in the absence of recessive deleterious alleles. This effect is very pronounced in the immuno-competence MHC gene complex [148,149]. Therefore, when genetic drift reduces the proportion of heterozygotes in the gene pool, the population as a whole is rendered less 'fit'. Aside from these potential direct negative effects of reduced genetic variability, a limiting responsiveness to novel environmental threats (climate change, pesticides, competition with invasive species, disease, predation *etc*.) might develop as well. Because of reduced adaptive potential, genetic effects act to diminish the reproductive capabilities of isolated populations, and thus their persistence. Overall, the effects of reduced genetic diversity are of grave concern to small populations. Not only do such effects limit the evolutionary potential of populations, but in many cases they are also directly and clearly correlated with reduced fitness [100], potentially perpetuating a downward spiral towards local extinction. It is tempting to propose the relatively simple solution of establishing links between isolated breeding assemblages to counteract the negative effects of genetic drift. For example, a management strategy involving artificial mixing of lineages was successfully utilised to address inbreeding in captive populations of the Puerto Rican crested toad *Peltophryne lemur* [150]. However, caution must be exercised before considering such artificial gene flow [151]. **Outbreeding depression**, causing a negative GFC, may arise due to the disruption of co-adapted gene complexes, either between genes or between genes and the local environment. Reduced fitness in outbred individuals has been reported from a diversity of plants and animals, including amphibians [89].

Acknowledgements

References and Notes

1. Wake, D.B.; Vredenburg, V.T. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc. Nat. Acad. Sci. USA* **2008**, *105*, 11466-11473.

2. Pimm, S.L.; Russell, G.J.; Gittleman, J.L.; Brooks, T.M. The future of biodiversity. *Science* **1995**, *269*, 347-350.

3. Chapin, F.S.; Zavaleta, E.S.; Eviner, V.T.; Naylor, R.L.; Vitousek, P.M.; Reynolds, H.L.; Hooper, D.U.; Lavorel, S.; Sala, O.E.; Hobbie, S.E.; Mack, M.C.; Diaz, S. Consequences of changing biodiversity. *Nature* **2000**, *405*, 234-242.

4. Schellnhuber, H.J.; Kokott, J.; Beese, F.O.; Fraedrich, K.; Klemmer, P.; Kruse-Graumann, L.; Neumann, C.; Renn, O.; Schulze, E.D.; Tilzer, M.; Velsinger, P.; Zimmermann, H. *World in Transition: Conservation and Sustainable use of the Biosphere*. Earthscan Publications Ltd: London, UK, 2001.

5. Stuart, S.N.; Chanson, J.S.; Cox, N.A.; Young, B.E.; Rodrigues, A.S.L.; Fischman, D.L.; Waller, R.W. Status and trends of amphibian declines and extinctions worldwide. *Science* **2004**, *306*, 1783-1786.

6. McCallum, M.L. Amphibian decline or extinction? Current declines dwarf background extinction rate. *J. Herpetol.* **2007**, *41*, 483-491.

7. Beebee, T.J.; Wilkinson, J.; Buckley, J. Amphibian declines are not uniquely high amongst the vertebrates: trend determination and the British perspective. *Diversity* **2009**, *1*, 67-88.

8. Wind, E. Effects of habitat fragmentation on amphibians: what do we know and where do we go from here? In *Proceedings of the Biology and Management of Species and Habitats at Risk*, Darling, L.M., Ed.; B.C. Ministry of Environment, Lands and Parks, Victoria B.C. and University College of the Cariboo, Kamloops B.C., 1999; pp. 885-894.

9. Cushman, S.A. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biol. Conserv.* **2006**, *128*, 231-240.

10. Gallant, A.L.; Klaver, R.W.; Casper, G.S.; Lannoo, M.J. Global rates of habitat loss and implications for amphibian conservation. *Copeia* **2007**, *4*, 967-979.

11. Gardner, T.A.; Barlow, J.; Peres, C.A. Paradox, presumption and pitfalls in conservation biology: the importance of habitat change for amphibians and reptiles. *Biol. Conserv.* **2007**, *138*, 166-179.

12. Carey, C.; Alexander, M.A. Climate change and amphibian declines: is there a link? *Divers. Distrib.* **2003**, *9*, 111-121.

13. Corn, P. S. Climate change and amphibians. *Anim. Biodivers. Conserv.* **2005**, *28*, 59-67.

14. Licht, L.E.; Grant, K.P. The effects of ultraviolet radiation on the biology of amphibians. *Amer. Zool.* **1997**, *37*, 137-145.

15. Weyrauch, S.L.; Grubb, T.C. Effects of the interaction between genetic diversity and UV-B radiation on wood frog fitness. *Biol. Conserv.* **2006**, *20*, 802-810.

16. Blaustein, A.R.; Kiesecker, J.M. Complexity in conservation: lessons from the global decline of amphibian populations. *Ecol. Lett.* **2002**, *5*, 597-608.

17. Kats, L.B.; Ferrer, R.P. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Divers. Distrib.* **2003**, *9*, 99-110.

18. Carey, C.; Bryant, C.J. Possible interrelations among environmental toxicants, amphibian development, and decline of amphibian populations. *Environ. Health Perspect.* **1995**, *103*, 13-17.

19. Marco, A.; Quilchano, C.; Blaustein, A.R. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific northwest, USA. *Environ. Toxicol. Chem.* **1999**, *18*, 2836-2839.

20. Hels, T.; Buchwald, E. The effect of road kills on amphibian populations. *Biol. Conserv.* **2001**, *99*, 331-340.

21. Gibbs, J.P.; Shriver, W.G. Can road mortality limit populations of pool-breeding amphibians? *Wetlands Ecol. Manag.* **2005**, *13*, 281-289.

22. Warkentin, I.G.; Bickford, D.; Sodhi, N.S.; Bradshaw, C.J.A. Eating frogs to extinction. *Conserv. Biol.* **2009**, *23*, 1056-1059.

23. Daszak, P.; Cunningham, A.A.; Hyatt, A.D. Infectious disease and amphibian population declines. *Divers. Distrib.* **2003**, *9*, 141-150.

24. James, T.Y.; Litvintseva, A.P.; Vilgalys, R.; Morgan, J.A.T.; Taylor, J.W.; Fisher, M.C.; Berger, L.; Weldon, C.; du Preez, L.; Longcore, J.E. Rapid global expansion of the fungal disease chytridiomycosis into declining and healthy amphibian populations. *PLoS Pathog.* **2009**, *5*, e1000458.

25. Rödder, D.; Kielgast, J.; Bielby, J.; Schmidtlein, S.; Bosch, J.; Garner, T.W.; Veith, M.; Walker, S.; Fisher, M.; Lötters, S. Global amphibian extinction risk assessment for the panzootic chytrid fungus. *Diversity* **2009**, *1*, 52-66.

26. Voyles, J.; Young, S.; Berger, L.; Campbell, C.; Voyles, W.F.; Dinudom, A.; Cook, D.; Webb, R.; Alford, R.A.; Skerratt, L.F.; Speare, R. Pathogenesis of chytridiomycosis, a cause of catastrophic amphibian declines. *Science* **2009**, *326*, 582-585.

27. Collins, J.P.; Storfer, A. Global amphibian declines: sorting the hypotheses. *Divers. Distrib.* **2003**, *9*, 89-98.

28. Blaustein, A.R.; Bancroft, B.A. Amphibian population declines: Evolutionary considerations. *Bioscience* **2007**, *57*, 437-444.

29. Pounds, J.A.; Bustamante, M.R.; Coloma, L.A.; Consuegra, J.A.; Fogden, M.P.L.; Foster, P.N.; La Marca, E.; Masters, K.L.; Merino-Viteri, A.; Puschendorf, R.; Ron, S.R.; Sanchez-Azofeifa, G.A.; Still, C.J.; Young, B.E. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **2006**, *439*, 161-167.

30. Kiesecker, J.M.; Blaustein, A.R. Synergism between UV-B radiation and a pathogen magnifies amphibian embryo mortality in nature. *Proc. Nat. Acad. Sci. USA* **1995**, *92*, 11049-11052.

31. Rohr, J.R.; Schotthoefer, A.M.; Raffel, T.R.; Carrick, H.J.; Halstead, N.; Hoverman, J.T.; Johnson, C.M.; Johnson, L.B.; Lieske, C.; Piwoni, M.D.; Schoff, P.K.; Beasley, V.R. Agrochemicals increase trematode infections in a declining amphibian species. *Nature* **2008**, *455*, 1235-1239.

32. Shaffer, M.L. Population viability analysis. *Conserv. Biol.* **1990**, *4*, 39-40.

33. Frankel, O.H.; Soulé, M.E. *Conservation and Evolution*; Cambridge University Press: Cambridge, UK, 1981.

34. Willi, Y.; Van Buskirk, J.; Hoffmann, A.A. Limits to the adaptive potential of small populations. *Ann. Rev. Ecol. Evol. Syst.* **2006**, *37*, 433-458.

35. Frankham, R.; Ballou, J.D.; Briscoe, D.A. *Introduction to Conservation Genetics*; Cambridge University Press: Cambridge, UK, 2002.

36. Funk, W.C.; Tallmon, D.A.; Allendorf, F.W. Small effective population size in the long-toed salamander. *Mol. Ecol.* **1999**, *8*, 1633-1640.

37. Beebee, T.J.C.; Griffiths, R.A. The amphibian decline crisis: a watershed for conservation biology? *Biol. Conserv.* **2005**, *125*, 271-285.

38. Schmeller, D.S.; Merila, J. Demographic and genetic estimates of effective population and breeding size in the amphibian *Rana temporaria*. *Conserv. Biol.* **2007**, *21*, 142-151.

39. Scribner, K.T.; Arntzen, J.W.; Burke, T. Effective number of breeding adults in *Bufo bufo* estimated from age-specific variation at minisatellite loci. *Mol. Ecol.* **1997**, *6*, 701-712.

40. Rowe, G.; Beebee, T.J.C. Reconciling genetic and demographic estimators of effective population size in the anuran amphibian *Bufo calamita*. *Conserv. Genet.* **2004**, *5*, 287-298.

41. Pechmann, J.H.K.; Scott, D.E.; Semlitsch, R.D.; Caldwell, J.P.; Vitt, L.J.; Gibbons, J.W. Declining amphibian populations—the problem of separating human impacts from natural fluctuations. *Science* **1991**, *253*, 892-895.

42. Richter, S.C.; Young, J.E.; Johnson, G.N.; Seigel, R.A. Stochastic variation in reproductive success of a rare frog, *Rana sevosa*: implications for conservation and for monitoring amphibian populations. *Biol. Conserv.* **2003**, *111*, 171-177.

43. Alford, R.A.; Richards, S.J. Global amphibian declines: a problem in applied ecology. *Ann. Rev. Ecol. Syst.* **1999**, *30*, 133-165.

44. Ficetola, G.F.; Padoa-Schioppa, E.; Wang, J.; Garner, T.W.J. Polygyny, census and effective population size in the threatened frog, *Rana latastei*. *Anim. Conserv.* **2009**, doi:10.1111/j.1469-1795.2009.00306.x.

45. Vucetich, J.A.; Waite, T.A. Erosion of heterozygosity in fluctuating populations. *Conserv. Biol.* **1999**, *13*, 860-868.

46. Cabe, P.R.; Page, R.B.; Hanlon, T.J.; Aldrich, M.E.; Connors, L.; Marsh, D.M. Fine-scale population differentiation and gene flow in a terrestrial salamander (*Plethodon cinereus*) living in continuous habitat. *Heredity* **2007**, *98*, 53-60.

47. Driscoll, D.A. Genetic structure of the frogs *Geocrinia lutea* and *Geocrinia rosea* reflects extreme population divergence and range changes, not dispersal barriers. *Evolution* **1998**, *52*, 1147-1157.

48. Dubois, A. Developmental pathway, speciation and supraspecific taxonomy in amphibians 1. Why are there so many frog species in Sri Lanka? *Alytes* **2004**, *22*, 19-37.

49. Sodhi, N.S.; Bickford, D.; Diesmos, A.C.; Lee, T.M.; Koh, L.P.; Brook, B.W.; Sekercioglu, C.H.; Bradshaw, C.J.A. Measuring the meltdown: drivers of global amphibian extinction and decline. *PLoS One* **2008**, *3*, Article No.: e1636.

50. Frankham, R. Relationship of genetic variation to population size in wildlife. *Conserv. Biol.* **1996**, *10*, 1500-1508.

51. Andersen, L.W.; Fog, K.; Damgaard, C. Habitat fragmentation causes bottlenecks and inbreeding in the European tree frog (*Hyla arborea*). *Proc. Roy. Soc. London Ser. B* **2004**, *271*, 1293-1302.

52. Rowe, G.; Beebee, T.J.C.; Burke, T. Microsatellite heterozygosity, fitness and demography in natterjack toads *Bufo calamita*. *Anim. Conserv.* **1999**, *2*, 85-92.

53. Marsh, D.M.; Trenham, P.C. Metapopulation dynamics and amphibian conservation. *Conserv. Biol.* **2001**, *15*, 40-49.

54. Hanski, I. Metapopulation dynamics. *Nature* **1998**, *396*, 41-49.

55. Fischer, M. Species loss after habitat fragmentation. *Trend. Ecol. Evol.* **2000**, *15*, 396-396.

56. Harrison, S.; Bruna, E. Habitat fragmentation and large-scale conservation: what do we know for sure? *Ecography* **1999**, *22*, 225-232.

57. Pimm, S.L.; Raven, P. Biodiversity—Extinction by numbers. *Nature* **2000**, *403*, 843-845.

58. Tilman, D.; May, R.M.; Lehman, C.L.; Nowak, M.A. Habitat destruction and the extinction depth. *Nature* **1994**, *371*, 65-66.

59. DiBattista, J.D. Patterns of genetic variation in anthropogenically impacted populations. *Conserv. Genet.* **2008**, *9*, 141-156.

60. Toro, M.A.; Caballero, A. Characterization and conservation of genetic diversity in subdivided populations. *Phil. Trans. Roy. Soc. B-Biol. Sci.* **2005**, *360*, 1367-1378.

61. Young, A.G.; Clarke, G.M. *Genetics, Demography and Viability of Fragmented Populations*; Cambridge University Press: Cambridge, UK, 2000.

62. Allentoft, M.E.; Siegismund, H.R.; Briggs, L.; Andersen, L.W. Microsatellite analysis of the natterjack toad (*Bufo calamita*) in Denmark: populations are islands in a fragmented landscape. *Conserv. Genet.* **2009**, *10*, 15-28.

63. Richter, S.C.; Crother, B.I.; Broughton, R.E. Genetic consequences of population reduction and geographic isolation in the critically endangered frog, *Rana sevosa*. *Copeia* **2009**, 801-808.

64. Arens, P.; van der Sluis, T.; van't Westende, W.P.C.; Vosman, B.; Vos, C.C.; Smulders, M.J.M. Genetic population differentiation and connectivity among fragmented Moor frog (*Rana arvalis*) populations in The Netherlands. *Landscape Ecol.* **2007**, *22*, 1489-1500.

65. Hitchings, S.P.; Beebee, T.J.C. Genetic substructuring as a result of barriers to gene flow in urban *Rana temporaria* (common frog) populations: implications for biodiversity conservation. *Heredity* **1997**, *79*, 117-127.

66. Lesbarreres, D.; Primmer, C.R.; Lode, T.; Merila, J. The effects of 20 years of highway presence on the genetic structure of *Rana dalmatina* populations. *Ecoscience* **2006**, *13*, 531-538.

67. Reh, W.; Seitz, A. The influence of land use on the genetic structure of populations of the common frog *Rana temporaria*. *Biol. Conserv.* **1990**, *54*, 239-249.

68. Vos, C.C.; Antonisse-De Jong, A.G.; Goedhart, P.W.; Smulders, M.J.M. Genetic similarity as a measure for connectivity between fragmented populations of the moor frog (*Rana arvalis*). *Heredity* **2001**, *86*, 598-608.

69. Spear, S.F.; Storfer, A. Landscape genetic structure of coastal tailed frogs (*Ascaphus truei*) in protected *vs.* managed forests. *Mol. Ecol.* **2008**, *17*, 4642-4656.

70. Curtis, J.M.R.; Taylor, E.B. The genetic structure of coastal giant salamanders (*Dicamptodon tenebrosus*) in a managed forest. *Biol. Conserv.* **2004**, *115*, 45-54.

71. Dixo, M.; Metzger, J.P.; Morgante, J.S.; Zamudio, K.R. Habitat fragmentation reduces genetic diversity and connectivity among toad populations in the Brazilian Atlantic Coastal Forest. *Biol. Conserv.* **2009**, *142*, 1560-1569.

72. Telles, M.P.de C.; Diniz-Filho, J.A.F.; Bastos, R.P.; Soares, T.N.; Guimaraes, L.D.; Lima, L.P. Landscape genetics of *Physalaemus cuvieri* in Brazilian cerrado: correspondence between population structure and patterns of human occupation and habitat loss. *Biol. Conserv.* **2007**, *139*, 37-46.

73. Burns, E.L.; Eldridge, M.D.B.; Houlden, B.A. Microsatellite variation and population structure in a declining Australian hylid *Litoria aurea*. *Mol. Ecol.* **2004**, *13*, 1745-1757.

74. Hazell, D. Frog ecology in modified Australian landscapes: a review. *Wildlife Res.* **2003**, *30*, 193-205.

75. Funk, W.C.; Blouin, M.S.; Corn, P.S.; Maxell, B.A.; Pilliod, D.S.; Amish, S.; Allendorf, F.W. Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Mol. Ecol.* **2005**, *14*, 483-496.

76. Garner, T.W.J.; Pearman, P.B.; Angelone, S. Genetic diversity across a vertebrate species' range: a test of the central-peripheral hypothesis. *Mol. Ecol.* **2004**, *13*, 1047-1053.

77. Beebee, T.J.C.; Rowe, G. Microsatellite analysis of natterjack toad *Bufo calamita* Laurenti populations: consequences of dispersal from a Pleistocene refugium. *Biol. J. Linn. Soc.* **2000**, *69*, 367-381.

78. Ficetola, G.F.; Garner, T.W.J.; De Bernardi, F. Genetic diversity, but not hatching success, is jointly affected by postglacial colonization and isolation in the threatened frog, *Rana latastei*. *Mol. Ecol.* **2007**, *16*, 1787-1797.

79. Blaustein, A.R.; Wake, D.B.; Sousa, W.P. Amphibian declines—judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conserv. Biol.* **1994**, *8*, 60-71.

80. Tallmon, D.A.; Funk, W.C.; Dunlap, W.W.; Allendorf, F.W. Genetic differentiation among Long-Toed Salamander (*Ambystoma macrodactylum*) populations. *Copeia* **2000**, 27-35.

81. Kraaijeveld-Smit, F.J.L.; Beebee, T.J.C.; Griffiths, R.A.; Moore, R.D.; Schley, L. Low gene flow but high genetic diversity in the threatened Mallorcan midwife toad *Alytes muletensis*. *Mol. Ecol.* **2005**, *14*, 3307-3315.

82. Spear, S.F.; Peterson, C.R.; Matocq, M.D.; Storfer, A. Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Mol. Ecol.* **2005**, *14*, 2553-2564.

83. Sinsch, U. Migration and orientation in anuran amphibians. *Ethol. Ecol. Evol.* **1990**, *2*, 65-79.

84. Rothermel, B.B.; Semlitsch, R.D. An experimental investigation of landscape resistance of forest *versus* old-field habitats to emigrating juvenile amphibians. *Conserv. Biol.* **2002**, *16*, 1324-1332.

85. Trenham, P.C.; Shaffer, H.B. Amphibian upland habitat use and its consequences for population viability. *Ecol. Appl.* **2005**, *15*, 1158-1168.

86. Smith, M.A.; Green, D.M. Dispersal and the metapopulation paradigm in amphibian ecology and conservation: are all amphibian populations metapopulations? *Ecography* **2005**, *28*, 110-128.

87. Phillips, B.L.; Shine, R. Spatial and temporal variation in the morphology (and thus, predicted impact) of an invasive species in Australia. *Ecography* **2006**, *29*, 205-212.

88. Bowne, D.R.; Bowers, M.A. Interpatch movements in spatially structured populations: a literature review. *Landscape Ecol.* **2004**, *19*, 1-20.

89. Sagvik, J.; Uller, T.; Olsson, M. Outbreeding depression in the common frog, *Rana temporaria*. *Conserv. Genet.* **2005**, *6*, 205-211.

90. Sjogren, P. Extinction and isolation gradients in metapopulations - the case of the pool frog (*Rana lessonae*). *Biol. J. Linn. Soc.* **1991**, *42*, 135-147.

91. Vos, C.C.; Chardon, J.P. Effects of habitat fragmentation and road density on the distribution pattern of the moor frog *Rana arvalis*. *J. Appl. Ecol.* **1998**, *35*, 44-56.

92. Carr, L.W.; Fahrig, L. Effect of road traffic on two amphibian species of differing vagility. *Conserv. Biol.* **2001**, *15*, 1071-1078.

93. Funk, W.C.; Greene, A.E.; Corn, P.S.; Allendorf, F.W. High dispersal in a frog species suggests that it is vulnerable to habitat fragmentation. *Biol. Lett.* **2005**, *1*, 13-16.

94. Crnokrak, P.; Roff, D.A. Inbreeding depression in the wild. *Heredity* **1999**, *83*, 260-270.

95. Hedrick, P.W.; Kalinowski, S.T. Inbreeding depression in conservation biology. *Ann. Rev. Ecol. Syst.* **2000**, *31*, 139-162.

96. Hansson, B.; Westerberg, L. On the correlation between heterozygosity and fitness in natural populations. *Mol. Ecol.* **2002**, *11*, 2467-2474.

97. DeWoody, Y.D.; DeWoody, J.A. On the estimation of genome-wide heterozygosity using molecular markers. *J. Hered.* **2005**, *96*, 85-88.

98. Balloux, F.; Amos, W.; Coulson, T. Does heterozygosity estimate inbreeding in real populations? *Mol. Ecol.* **2004**, *13*, 3021-3031.

99. Lesbarreres, D.; Primmer, S.R.; Laurila, A.; Merila, J. Environmental and population dependency of genetic variability-fitness correlations in *Rana temporaria*. *Mol. Ecol.* **2005**, *14*, 311-323.

100. Reed, D.H.; Frankham, R. Correlation between fitness and genetic diversity. *Conserv. Biol.* **2003**, *17*, 230-237.

101. Coltman, D.W.; Slate, J. Microsatellite measures of inbreeding: a meta-analysis. *Evolution* **2003**, *57*, 971-983.

102. Chapman, J.R.; Nakagawa, S.; Coltman, D.W.; Slate, J.; Sheldon, B.C. A quantitative review of heterozygosity-fitness correlations in animal populations. *Mol. Ecol.* **2009**, *18*, 2746-2765.

103. Gower, D.J.; Wilkinson, M. Conservation biology of caecilian amphibians. *Conserv. Biol.* **2005**, *19*, 45-55.

104. Nace, G.W.; Richards, C.M.; Asher, J.H. Parthenogenesis and genetic variability.1. Linkage and inbreeding estimations in the frog, *Rana pipiens*. *Genetics* **1970**, *66*, 349-368.

105. Zeisset, I.; Beebee, T.J.C. Population genetics of a successful invader: the marsh frog *Rana ridibunda* in Britain. *Mol. Ecol.* **2003**, *12*, 639-646.

106. Samollow, P.B.; Soule, M.E. A case of stress related heterozygote superiority in nature. *Evolution* **1983**, *37*, 646-649.

107. Pierce, B.A.; Mitton, J.B. Allozyme heterozygosity and growth in the tiger salamander, *Ambostyma tigrinum*. *J. Hered.* **1982**, *73*, 250-253.

108. Schmeller, D.S.; Schregel, J.; Veith, M. The importance of heterozygosity in a frog's life. *Naturwissenschaften* **2007**, *94*, 360-366.

109. Hitchings, S.P.; Beebee, T.J.C. Loss of genetic diversity and fitness in Common Toad (*Bufo bufo*) populations isolated by inimical habitat. *J. Evol. Biol.* **1998**, *11*, 269-283.

110. Johansson, M.; Primmer, C.R.; Merila, J. Does habitat fragmentation reduce fitness and adaptability? A case study of the common frog (*Rana temporaria*). *Mol. Ecol.* **2007**, *16*, 2693-2700.

111. Halverson, M.A.; Skelly, D.K.; Caccone, A. Inbreeding linked to amphibian survival in the wild but not in the laboratory. *J. Hered.* **2006**, *97*, 499-507.

112. Whitehurst, P.H.; Pierce, B.A. The relationship between allozyme variation and life history traits of the spotted chorus frog *Pseudacris clarkii. Copeia* **1991**, 1032-1039.

113. Rowe, G.; Beebee, T.J.C. Population on the verge of a mutational meltdown? Fitness costs of genetic load for an amphibian in the wild. *Evolution* **2003**, *57*, 177-181.

114. Williams, R.N.; Bos, D.H.; Gopurenko, D.; DeWoody, J.A. Amphibian malformations and inbreeding. *Biol. Lett.* **2008**, *4*, 549-552.

115. Mitton, J.B.; Carey, C.; Kocher, T.D. The relation of enzyme heterozygosity to standard and active oxygen-consumption and body size of tiger salamanders, *Ambystoma tigrinum. Physiol. Zool.* **1986**, *59*, 574-582.

116. Rowe, G.; Beebee, T.J.C. Intraspecific competition disadvantages inbred natterjack toad (*Bufo calamita*) genotypes over outbred ones in a shared pond environment. *J. Anim. Ecol.* **2005**, *74*, 71-76.

117. McAlpine, S. Genetic heterozygosity and reproductive success in the green treefrog, *Hyla cinerea. Heredity* **1993**, *70*, 553-558.

118. Slate, J.; David, P.; Dodds, K.G.; Veenvliet, B.A.; Glass, B.C.; Broad, T.E.; McEwan, J.C. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* **2004**, *93*, 255-265.

119. Rowe, G.; Beebee, T.J.C. Fitness and microsatellite diversity estimates were not correlated in two outbred anuran populations. *Heredity* **2001**, *87*, 558-565.

120. Slate, J.; Pemberton, J. Does reduced heterozygosity depress sperm quality in wild rabbits (*Oryctolagus cuniculus*)? *Curr. Biol.* **2006**, *16*, R790-R791.

121. Baer, B.; Schmid-Hempel, P. Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. *Nature* **1999**, *397*, 151-154.

122. Acevedo-Whitehouse, K.; Gulland, F.; Greig, D.; Amos, W. Disease susceptibility in California sea lions. *Nature* **2003**, *422*, 35-35.

123. Hughes, W.O.H.; Boomsma, J.J. Genetic diversity and disease resistance in leaf-cutting ant societies. *Evolution* **2004**, *58*, 1251-1260.

124. Spielman, D.; Brook, B.W.; Briscoe, D.A.; Frankham, R. Does inbreeding and loss of genetic diversity decrease disease resistance? *Conserv. Genet.* **2004**, *5*, 439-448.

125. Daszak, P. Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* **2000**, *287*, 1756-1756.

126. Pearman, P.B.; Garner, T.W.J. Susceptibility of Italian agile frog populations to an emerging strain of Ranavirus parallels population genetic diversity. *Ecol. Lett.* **2005**, *8*, 401-408.

127. Parris, M.J. Hybrid response to pathogen infection in interspecific crosses between two amphibian species (Anura: Ranidae). *Evol. Ecol. Res.* **2004**, *6*, 457-471.

128. Bridges, C.M.; Semlitsch, R.D. Genetic variation in insecticide tolerance in a population of southern leopard frogs (*Rana sphenocephala*): Implications for amphibian conservation. *Copeia* **2001**, 7-13.

129. Pierce, B.A.; Sikand, N. Variation in acid tolerance of Connecticut wood frogs—genetic and maternal effects. *Can. J. Zool.* **1985**, *63*, 1647-1651.

130. Semlitsch, R.D.; Bridges, C.M.; Welch, A.M. Genetic variation and a fitness tradeoff in the tolerance of gray treefrog (*Hyla versicolor*) tadpoles to the insecticide carbaryl. *Oecologia* **2000**, *125*, 179-185.

131. Laurila, A.; Karttunen, S.; Merila, J. Adaptive phenotypic plasticity and genetics of larval life histories in two *Rana temporaria* populations. *Evolution* **2002**, *56*, 617-627.

132. Laugen, A.T.; Laurila, A.; Rasanen, K.; Merila, J. Latitudinal countergradient variation in the common frog (*Rana temporaria*) development rates—evidence for local adaptation. *J. Evol. Biol.* **2003**, *16*, 996-1005.

133. Weitere, M.; Tautz, D.; Neumann, D.; Steinfartz, S. Adaptive divergence *vs.* environmental plasticity: tracing local genetic adaptation of metamorphosis traits in salamanders. *Mol. Ecol.* **2004**, *13*, 1665-1677.

134. Blaustein, A.R.; Edmond, B.; Kiesecker, J.M.; Beatty, J.J.; Hokit, D.G. Ambient ultraviolet-radiation causes mortality in salamander eggs. *Ecol. Appl.* **1995**, *5*, 740-743.

135. Hays, J.B.; Blaustein, A.R.; Kiesecker, J.M.; Hoffman, P.D.; Pandelova, I.; Coyle, D.; Richardson, T. Developmental responses of amphibians to solar and artificial UVB sources: A comparative study. *Photochem. Photobiol.* **1996**, *64*, 449-456.

136. Anzalone, C.R.; Kats, L.B.; Gordon, M.S. Effects of solar UV-B radiation on embryonic development in *Hyla cadaverina*, *Hyla regilla*, and *Taricha torosa*. *Conserv. Biol.* **1998**, *12*, 646-653.

137. Brook, B.W.; Tonkyn, D.W.; Q'Grady, J.J.; Frankham, R. Contribution of inbreeding to extinction risk in threatened species. *Conserv. Ecol.* **2002**, *6*.

138. Spielman, D.; Brook, B.W.; Frankham, R. Most species are not driven to extinction before genetic factors impact them. *Proc. Nat. Acad. Sci. USA* **2004**, *101*, 15261-15264.

139. Lande, R. Genetics and demography in biological conservation. *Science* **1988**, *241*, 1455-1460.

140. Hedrick, P. W. *Genetics of Populations*. Jones & Bartlett Publishers: Boston, 2004.

141. Kimura, M.; Ohta, T. *Theoretical Aspects of Population Genetics*; Princeton University Press: New Jersey, USA, 1971.

142. Lacy, R.C. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection and population subdivision. *Conserv. Biol.* **1987**, *1*, 143-158.

143. Keller, L.F.; Waller, D.M. Inbreeding effects in wild populations. *Trend. Ecol. Evol.* **2002**, *17*, 230-241.

144. Caughley, G. Directions in conservation biology. *J. Anim. Ecol.* **1994**, *63*, 215-244.

145. Hedrick, P.W. Purging inbreeding depression and the probability of extinction - full sib mating. *Heredity* **1994**, *73*, 363-372.

146. Leberg, P.L.; Firmin, B.D. Role of inbreeding depression and purging in captive breeding and restoration programmes. *Mol. Ecol.* **2008**, *17*, 334-343.

147. Charlesworth, B. The effect of synergistic epistasis on the inbreeding load. *Genet. Res.* **1998**, *71*, 85-89.

148. Hughes, A.L.; Nei, M. Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. *Nature* **1988**, *335*, 167-170.

149. Radwan, J.; Biedrzycka, A.; Babik, W. Does reduced MHC diversity decrease viability of vertebrate populations? *Biol. Conserv.* **2010**, in press.

150. Beauclerc, K.; Johnson, B.; White, B. Genetic rescue of an inbred captive population of the critically endangered Puerto Rican crested toad (*Peltophryne lemur*) by mixing lineages. *Conserv. Genet. Available online*, doi 10.1007/s10592-10008-19782-z.

151. Edmands, S. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Mol. Ecol.* **2007**, *16*, 463-475.

152. Edenhamn, P.; Hoggren, M.; Carlson, A. Genetic diversity and fitness in peripheral and central populations of the European tree frog *Hyla arborea*. *Hereditas* **2000**, *133*, 115-122.

153. McAlpine, S.; Smith, M.H. Genetic correlates of fitness in the green treefrog, *Hyla cinerea*. *Herpetologica* **1995**, *51*, 393-400.

154. Parris, M.J.; Laird, C.W.; Semlitsch, R.D. Differential predation on experimental populations of parental and hybrid leopard frog (*Rana blairi* and *Rana sphenocephala*) larvae. *J. Herpetol.* **2001**, *35*, 479-485.

155. Parris, M.J. Hybridization in leopard frogs (*Rana pipiens complex*): larval fitness components in single-genotype populations and mixtures. *Evolution* **1999**, *53*, 1872-1883.

156. Parris, M.J. Experimental analysis of hybridization in leopard frogs (Anura: Ranidae): larval performance in desiccating environments. *Copeia* **2000**, *2000*, 11-19.

157. Parris, M.J. Hybridization in leopard frogs (*Rana pipiens complex*): terrestrial performance of newly metamorphosed hybrid and parental genotypes in field enclosures. *Can. J. Zool.* **2001**, *79*, 1552-1558.

158. Parris, M.J. High larval performance of leopard frog hybrids: effects of environment-dependent selection. *Ecology* **2001**, *82*, 3001-3009.

159. Planade, B.; Lena, J.-P.; Li, H.; Plenet, S.; Guegan, J.-F.; Thomas, F.; Hurtrez-Bousses, S.; Renaud, F.; Joly, P. Tracking a heterosis effect in the field: tadpole resistance to parasites in the water frog hybridogenetic complex. *Parasitology* **2009**, *136*, 1003-1013.

160. Wright, M.F.; Guttman, S.I. Lack of an association between heterozygosity and growth rate in the wood frog, *Rana sylvatica*. *Can. J. Zool.* **1995**, *73*, 569-575.

161. Lesbarreres, D.; Schmeller, D.S.; Primmer, C.R.; Merila, J. Genetic variability predicts common frog (*Rana temporaria*) size at metamorphosis in the wild. *Heredity* **2007**, *99*, 41-46.

Appendix 1. An overview of 34 studies that examine a link between genetics and fitness in amphibians.

Taxon	Reference	Molecular markers	Fitness trait	GFC	Comments
Ambystoma tigrinum	Mitton *et al.* [115]^	8 allozymes	oxygen consumption larvae growth	yes yes	
Ambystoma tigrinum	Pierce & Mitton [107]^	7 allozymes	early larvae growth (in wild/lab) late larvae growth (in wild)	yes*/yes no*	
Ambystoma tigrinum	Williams *et al.* [114]^	6 microsatellites and mtDNA	malformations in adults malformations in larvae	no* no*	
Bufo boreas	Samollow & Soule [106]^	9 allozymes	survival during metamorphosis survival during winter	no* yes*	
Bufo bufo	Hitchings & Beebee [109]^	27 allozymes	larvae malformation larvae survival	yes yes	
Bufo calamita	Rowe & Beebee [113]	inbred vs outbred population	larvae survival larvae growth	yes* yes*	Higher survival in outbred population
Bufo calamita	Rowe & Beebee [116]	inbred vs outbred population	larvae competitive potential	yes*	Outbred larvae superior in competition
Bufo calamita	Rowe *et al.* [52]^	8 microsatellites	larvae survival larvae growth	no yes	
Bufo calamita & Rana temporaria	Rowe & Beebee [119]^	5 and 7 microsatellites	larvae growth larvae development rate	no no	
Hyla arborea	Andersen *et al.* [51]	12 microsatellites	larvae survival	yes	Lower fitness in inbred populations
Hyla arborea	Edenhamn *et al.* [152]	18 allozymes (only one variable)	hatching success larvae survival	no no	High fitness despite very low genetic variation
Hyla cinerea	McAlpine [117]^	8 allozymes (in parents)	clutch size hatching success parent body size parent mating success	yes yes no* no*	
Hyla cinerea	McAlpine & Smith [153]^	8 allozymes	mating success adult survival	no* no*	Heterozygosity in one locus did affect fitness though
Pseudacris clarkii	Whitehurst & Pierce [112]^	9 allozymes	larvae growth larvae development rate	no yes	
Rana blairi & Rana sphenocephala	Parris [127]	hybridization experiment	larvae suscept. to chytrid fungus	yes	F1 hybrids more suscept. than parental genotypes
Rana blairi & Rana sphenocephala	Parris *et al.* [154]	hybridization experiment	larvae suscept. to predation	no	F1 hybrids and parents performed equally well
Rana blairi & Rana sphenocephala	Parris *et al.* [155-158]	hybridization experiments	various larvae/juvenile fitness traits	diff. results	Various hybridization experiments
Rana latastei	Ficetola *et al.* [78]^	6 microsatellites	hatching success	yes	

Taxon	Reference	Molecular markers	Fitness trait	GFC	Comments
Rana latastei	Pearman & Garner [126]^	6 microsatellites	*Ranavirus* and larvae survival	yes	
Rana lessonae	Sjogren [90]	not assessed	hatching success	no*	High fitness despite sampling isolated edge populations
Rana lessonae & Rana esculenta	Planade *et al.* [159]	hybridization experiment	tadpole parasite load	no	Hybrids and parental strains had same parasite load
Rana perezi	Schmeller *et al.* [108]^	13 allozymes	age (adult survival)	yes*	
Rana pipiens	Nace *et al.* [104]	gynogenetic experiment	postmetamorphic survival	yes	Inbred, (uniparental) strains had reduced fitness
Rana ridibunda	Zeisset & Beebee [105]	14 RAPD's and 5 microsatellites	larvae survial larvae growth	no no	High fitness despite known founder effect
Rana sylvatica	Halverson *et al.* [111]^	10 microsatellites	larvae survival (in wild/lab) larvae growth (in wild/lab) larvae development rate (in wild/lab)	yes*/no no*/no no*/no	
Rana sylvatica	Weyrauch & Grubb [15]^	25 RAPD 's	hatching success larvae survival larvae malformation larvae suscept. to UV-B	yes yes yes yes	
Rana sylvatica	Wright & Guttman [160]^	7 allozymes	larvae growth	no*	
Rana temporaria	Hitchings & Beebee [65]	16 allozymes	larvae survial larvae malformation	yes yes	Reduced fitness in fragmented urban populations
Rana temporaria	Johansson *et al.* [110]^	7 microsatellites	larvae survival larvae growth	yes yes	
Rana temporaria	Lesbarreres *et al.* [99]^	8 microsatellites	larvae survival larvae growth larvae development rate	yes yes yes	
Rana temporaria	Lesbarreres *et al.* [161]^	8 microsatellites	larvae growth larvae development rate	yes* no*	
Rana temporaria	Sagvik *et al.* [89]	outbreeding experiment	larvae growth larvae malformation hatching success	yes yes no	Outbreeding reduces fitness: a negative GFC

^ = The study provides a direct measure of genetic diversity and correlates it against fitness (a true GFC study)

* = Fitness was measured in wild populations

Clarifying comments are provided when the results refer to a GFC-*like* study rather than a direct correlation between genetic diversity and fitness