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The relevance of time series in molecular ecology and conservation biology

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ABSTRACT

The genetic structure of a species is shaped by the interaction of contemporary and historical factors. Analyses of individuals from the same population sampled at different points in time can help to disentangle the effects of current and historical forces and facilitate the understanding of the forces driving the differentiation of populations. The use of such time series allows for the exploration of changes at the population and intraspecific levels over time. Material from museum collections plays a key role in understanding and evaluating observed population structures, especially if large numbers of individuals have been sampled from the same locations at multiple time points. In these cases, changes in population structure can be assessed empirically. The development of new molecular markers relying on short DNA fragments (such as microsatellites or single nucleotide polymorphisms) allows for the analysis of long-preserved and partially degraded samples. Recently developed techniques to construct genome libraries with a reduced complexity and next generation sequencing and their associated analysis pipelines have the potential to facilitate marker development and genotyping in non-model species. In this review, we discuss the problems with sampling and available marker systems for historical specimens and demonstrate that temporal comparative studies are crucial for the estimation of important population genetic parameters and to measure empirically the effects of recent habitat alteration. While many of these analyses can be performed with samples taken at a single point in time, the measurements are more robust if multiple points in time are studied. Furthermore, examining the effects of habitat alteration, population declines, and population bottlenecks is only possible if samples before and after the respective events are included.

Key words: adaptation, differentiation, effective population size, habitat history, population bottleneck, population fluctuations, population history, random genetic drift, selection.

CONTENTS

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I. INTRODUCTION

Populations can undergo strong fluctuations in size from one generation to the next. Usually these changes have a relatively small effect on the genetic composition of the population. Yet, depending on the population size and the cause of the fluctuation a significant change in the genetic composition of species can occur in a single generation. Therefore, information about a population collected from single points in time often yields an incomplete picture of the historical and ongoing biological processes influencing populations (Crispo & Chapman, 2009; Husemann *et al.*, 2012). Especially when the impacts of natural or anthropogenic events, which took place at a specific time point, are studied, only samples taken before and after the event may provide the information needed to understand the effects on the population.

Many studies have documented the genetic impact of population bottlenecks as a result of overharvesting and habitat destruction (e.g. Hauser *et al.*, 2002; Frankham, 2005), the differentiation of populations in response to limited connectivity and restricted gene flow (e.g. Danley *et al.*, 2000; Husemann *et al.*, 2012), the impact of introduced invasive species on native species (e.g. Ficetola, Bonin & Miaud, 2008; Ray *et al.*, 2012) and species responses to climate change (e.g. Ayre & Hughes, 2004; Chaloupka, Kamezaki & Limpus, 2008). However, all of these studies draw conclusions based on data collected from a single time point. While studies have suggested that single-year samplings are sufficient to provide a good estimate of the genetic composition of a population (see Gomaa *et al.*, 2011), multiple sampling points can be used to explore empirically the demographic history of populations and document the persistence of population structures (e.g. in naturally fragmented habitats). Temporal population genetic studies can quantify the effects of natural and anthropogenic factors on populations and generate robust estimates of their effective population sizes. In addition, temporal designs can be used to test for the loss of genetic diversity, or to show an increase in population differentiation as a result of increasing population isolation and/or lower effective population sizes (e.g. Harper, Maclean & Goulson, 2006; Crispo & Chapman, 2009). The vast amount of biological material stored in museum collections in combination with advanced DNA sequencing techniques makes it possible to study the intraspecific effects of environmental and population changes over time (Luikart *et al.*, 2003). Furthermore, the combination of whole-genome scans using next generation sequencing (NGS) and temporal population samplings allows the identification of changes in selective pressures over generations (Nielsen *et al.*, 2009; Allendorf, Hohenlohe & Luikart, 2010; Gompert *et al.*, 2010; Hohenlohe *et al.*, 2010; Stapley *et al.*, 2010). While studies focusing on population responses to environmental conditions have often been carried out *ex situ* in experimental situations with artificial selective regimes (Ball *et al.*, 2000; Bijlsma, Bundgaard & Boerema, 2000; Reed, Briscoe & Frankham, 2002; Kristensen, Loeschke & Hoffmann, 2008), the use of time series may allow researchers to study the impacts of anthropogenic disturbance and large-scale changes of environmental conditions (e.g. climate, nitrogen loads) to understand whether taxa or local populations have the genetic diversity required to adapt to future environmental changes within relatively short time periods.

In this review, we explore the potential biological materials, marker systems and associated limitations for time-series studies. We discuss the advantages of analysing time series in molecular ecology and conservation biology (*i*) to estimate effective population size and the impact of random genetic drift, (*ii*) to explore the demographic history of populations (e.g. population fluctuations and population bottlenecks), and (*iii*) to study the impacts of changed habitat features and the relevance of habitat histories on inter- and intraspecific levels of the genetic structure.

II. SUITABILITY OF SAMPLES AND MARKERS

Analyses of populations sampled at multiple points in time are becoming increasingly relevant in the field of modern population biology, especially in population genetics and population genomics (e.g. Wandeler, Hoeck & Keller, 2007; Nielsen & Hansen, 2008; Gomaa *et al.*, 2011). However, museum collections rarely harbour sufficient numbers of suitable samples. Such samples need to be collected from the same generation and the same location to avoid unaccounted structure in the data. In addition, the sample needs to be stored in a manner such that the DNA is preserved and easily extracted (Nielsen & Hansen, 2008). Therefore, studies need to be planned according to the historical material available. The historical material should be located, DNA should be isolated and markers tested. Testing the markers is particularly important since even in cases where vast amounts of samples are available genotyping may not be possible. Contemporary sampling should only be performed after these preparations have been accomplished.

The DNA quality of historical samples strongly depends on the way organisms were collected, preserved and stored as well as on the age of these samples (Dean & Ballard, 2004). Some chemicals such as formalin and ethyl acetate can degrade DNA (Dillon, Austin & Bartowsky, 1996; Schander & Halanych, 2003). Storing samples frozen or in high concentrations of ethanol can be costly and time-consuming and their storage requires space and organization. While many universities and museums have established cryobanks (Lermen *et al.*, 2009), most samples, especially those interesting for studies addressing the genetic diversity before environmental changes took place, have been prepared conventionally by pinning and air-drying or curing (animals), or mounted on paper (in the case of annual herbaceous plant species). These methods generally preserve samples in a way that allows additional morphometric comparisons which continue to be important ancillary information for population genetic data sets. Given the persistent importance of morphological methods and museum policies on destructive sampling, minimally invasive

and non-destructive DNA extraction protocols have been developed in order to minimize the damage to specimens while maximizing the DNA yield (Mundy, Unitt & Woodruff, 1997; Gilbert *et al.*, 2007, Tagliavia *et al.*, 2011).

Despite the development of new DNA isolation techniques, the variable and often highly degraded DNA from historical samples limits the choice of genetic markers available for population genetic analyses. Some genetic techniques, especially methods based on protein and RNA molecules, require very specific sample storage conditions and are generally not applicable to historical samples. By contrast, DNA can be well preserved in old biological materials, and small fragments of DNA have successfully been amplified from samples 100 to 100000 years old (e.g. Hofreiter *et al.*, 2002; Strange, Knoblett & Griswold, 2009; Hoeck *et al.*, 2010). However, the unsuitable storage of samples often leads to degradation so that only small fragments of DNA are available for analyses. This again limits the genetic markers that can be reliably genotyped for population analyses (Wandeler *et al.*, 2007; Nielsen & Hansen, 2008). Methods which are based on the analysis of fragment polymorphisms, such as random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP) and amplified fragment length polymorphisms (AFLP), are generally less suitable since highly degraded DNA can lead to misleading results due to homoplasy in these types of markers. The sequencing of larger genes or gene fragments can also be very difficult for degraded samples. In degraded samples, rarely are large genes left intact for sequencing and often multiple primer pairs have to be used to obtain the complete targeted fragment. For sequence analyses mitochondrial DNA (mtDNA) is preferred over nuclear DNA (nuDNA) because mtDNA occurs in higher copy numbers. Yet, mtDNA sequences are unable to detect recombination. As a result, nuclear markers are also desirable in population studies. Given the fragmented nature of degraded nuDNA samples, methods targeting small fragments of DNA are preferred. Here, generally two types of markers are most commonly used: microsatellites and single nucleotide polymorphisms (SNPs). These high-resolution markers are suitable for detecting genetic changes over short temporal and restricted spatial scales and are therefore frequently used in landscape and conservation genetics (Selkoe & Toonen, 2006). Little is known about the evolution of microsatellites, making it difficult to employ suitable evolutionary models during the analyses (Ellegren, 2004; Takezaki & Nei, 2009). By contrast, the bi-allelic SNPs are less variable compared to microsatellites, and represent the most common type of polymorphism in the genome. Due to its simplicity and broad range of applications, this marker system has become increasingly popular recently and is considered the marker of the future in population genetics (Morin, Luikert & Wayne, 2004).

The recent development of next generation sequencing (NGS) techniques facilitates the discovery and typing of large numbers of genes and gene fragments (Baird *et al.*, 2008; Davey & Blaxter, 2010; Davey *et al.*, 2011; Ekblom & Galindo, 2011; Hohenlohe *et al.*, 2011). Most NGS methods employed in population genomics use genome reduction techniques including restriction digests and fragment size selection (Gompert *et al.*, 2010; Hohenlohe *et al.*, 2011). This allows for the simultaneous selective amplification of the desired number of loci across a number of specimens. Dozens of individuals can be barcoded and then pooled within runs on a NGS platform. However, often a two-stage approach is employed where NGS methods are used only for marker discovery (e.g. Davey *et al.*, 2011; De Pristo *et al.*, 2011; Seeb *et al.*, 2011). In a second step suitable markers are chosen from the large sets discovered by NGS and are then genotyped using quantitative PCR-based approaches, high-resolution melting (HRM) curve methods or Sanger sequencing. In the future, the costs of SNP genotyping in non-model organisms will decline further and will allow for the detection of large numbers of SNPs to examine population genetic structures even over many generations (Allendorf *et al.*, 2010).

However, due to the above-mentioned limitations in sample availability one has to be aware that temporal studies will always be limited to relatively few species for which suitable material is available. Such flagship species will have to serve as representatives for other organisms with similar ecology and life histories.

III. EFFECTIVE POPULATION SIZE AND RANDOM GENETIC DRIFT

In populations which are geographically isolated and where gene flow is low or lacking, genetic drift can be one of the main evolutionary forces driving divergence. The effect of genetic drift is largely determined by the effective population size (N_e). Small populations are generally more vulnerable to random processes than large populations. By contrast, drift is thought to play a minor role in large and interconnected populations because immigration and emigration processes balance differentiation and the emergence of private alleles. Populations with a large effective population size can rapidly respond to selection pressures while smaller populations may lack the necessary genetic diversity. However, generally it is difficult to determine the force having the strongest impact on a species or a population.

One way of inferring the relative impact of drift *versus* selection is to quantify N_e (Franklin & Frankham, 1998). This is because the relative contribution of drift or selection is a function of N_e and the selection coefficient. In general, neutral alleles are governed by drift and non-neutral variants by selection. However, since selection is more effective in large populations where random events (drift) have a smaller impact, there is a threshold at which non-neutral alleles become effectively neutral and thus governed by drift. This threshold is given by the equation $4N_e s = 1$, where s is the selection coefficient. Thus, in very small populations, even deleterious alleles with high selection coefficients may become fixed and reduce the fitness of the population. This, in turn, may lead to a mutational meltdown in which

the population size continues to decline leading to the fixation of more deleterious alleles which then causes a further decline in population size and so on (Lynch *et al.*, 1993).

Temporal studies are effective at estimating N_e by examining the change in allele frequencies through time. Stable allele frequencies reflect a large N_e; fluctuating allele frequencies on the other hand indicate a small N_e. While not the only method, time series can be used to estimate N_e and yield the most robust results (Barker, 2011).

Several studies have shown that drift can result in significant population divergence over different time frames. A study by Hoeck *et al.* (2010) showed that the degree of population divergence through drift strongly depends on habitat size which correlated with N_e. The smaller a population, the higher the degree of population divergence it experienced over the study period of approximately 200 generations. Similarly strong genetic drift was shown by Harper *et al.* (2006) for a butterfly species. Here, elevated drift was the result of a dramatic population reduction due to the decline in the butterfly's trophic resource. Other studies yielded similar results of temporal instability and significant changes of allele frequencies over different time scales (e.g. Heath *et al.*, 2002; Morris, Baucom & Cruzan, 2002; Breinholt *et al.*, 2009; Griffith *et al.*, 2009). Analyses of the Yellowstone grizzly bear (*Ursus arctos*) show a strong decline in genetic diversity between 1912 and 1981 in addition to reduced individual viability. The decline in the population's overall fitness may be a consequence of this population's genetic impoverishment and lower heterozygosity (Miller & Waits, 2003).

However, changes in population structure need not always result in changes in intraspecific genetic variability, but instead can lead to strong changes in genetic differentiation. For example, the genetic differentiation of *Erysimum cheiranthoides*, an annual plant common on stony river banks, increased threefold from 2005 to 2007, while the genetic diversity remained fairly constant through the years (Honnay *et al.*, 2009). High gene flow rates between the 16 studied populations and the relatively recent origin of the metapopulation structure may explain why recurrent extinction and colonization have not caused a decrease of genetic diversity. The authors argue that persistent seed banks play an important role in both maintaining the genetic diversity and in structuring the population after a moderate flooding event in 2007.

In contrast to these examples in which genetic diversity decreased and/or genetic differentiation increased from past to present, other studies showed temporal stability of population structures without significant shifts over time. Within a brown trout (*Salmo trutta*) population, genetic diversity and population structure experienced little change over a period of 20 years (Palm *et al.*, 2003). This example is in agreement with data obtained for the leopard frog, (*Rana pipiens*), where five populations were studied over 22–30 years (equivalent to 11–15 generations). The data indicate stable and very large effective population sizes and temporal stability of its genetic structure (Hoffman, Schueler & Blouin, 2004). These studies highlight that extant genetic structuring is strongly affected by past population dynamics which has a direct impact on genetic drift and gene flow.

IV. EFFECT OF POPULATION BOTTLENECKS

It is well known that demographic changes have the strongest impact on a population's genetic diversity (Frankham, Ballou & Briscoe, 2004), and temporal molecular analyses represent powerful tools to analyse these changes. Of the many ways in which a population can experience a demographic change, population bottlenecks produce the greatest genetic change due to genetic drift. In this case population sizes are drastically reduced and only a subset of the original diversity of a population is maintained. A textbook example was provided by Bouzat, Paige & Lewin (1998) who studied a population of the greater prairie chicken (*Tympanuchus cupido*) over a period of 30 years. They detected a large proportion of alleles which were exclusively found in historical samples but were absent in recently collected wild individuals. The authors coined the term 'ghost alleles' for these variants which are exclusively found in old sampling material, but have vanished in contemporary populations (Bouzat *et al.*, 1998). They argue that these ghost alleles disappeared due to strong population fluctuations and subsequent population bottlenecks. The reduction of genetic diversity in this example was significantly correlated with a decline in the population size (due to habitat loss) and finally caused a decrease in individual fitness (see also Hansson & Westerberg, 2002; Reed & Frankham, 2003; Leimu *et al.*, 2006). Similar trends of reductions in genetic diversity over generations in the wake of habitat transformation and associated reduced population sizes have also been found in other animal and plant species (e.g. Groombridge *et al.*, 2000; Harper *et al.*, 2006). When comparing seedlings of the highly endangered tree *Vateriopsis seychellarum*, endemic to the Seychelles, collected in pre- and post-fragmentation populations, the genetic data show a severe decline in genetic diversity together with an increase in genetic differentiation. The authors explain these effects as a consequence of the rapid reduction in the number of trees and low gene flow rates among local populations (Finger *et al.*, 2012).

The detection of ghost alleles in historical samples collected in a population need not necessarily imply a reduction in the total number of alleles or past population bottlenecks. The violet copper butterfly (*Lycaena helle*) has been geographically restricted to small and isolated habitats at higher elevations in the Middle Mountains of Central Europe since the postglacial warming. A comparison of its recent genetic diversity with individuals collected 15 years previously identified strong shifts in allele frequencies, the vanishing of many alleles (i.e. the existence of ghost alleles), but a relatively stable count in the total number of alleles over generations – despite its existence in rather small and isolated populations (Habel *et al.*, 2011) (Fig. 1). This is in accordance with a study by Harper *et al.* (2006) which showed large changes in allele frequencies but a stable number of alleles in the adonis blue butterfly (*Polyommatus bellargus*),

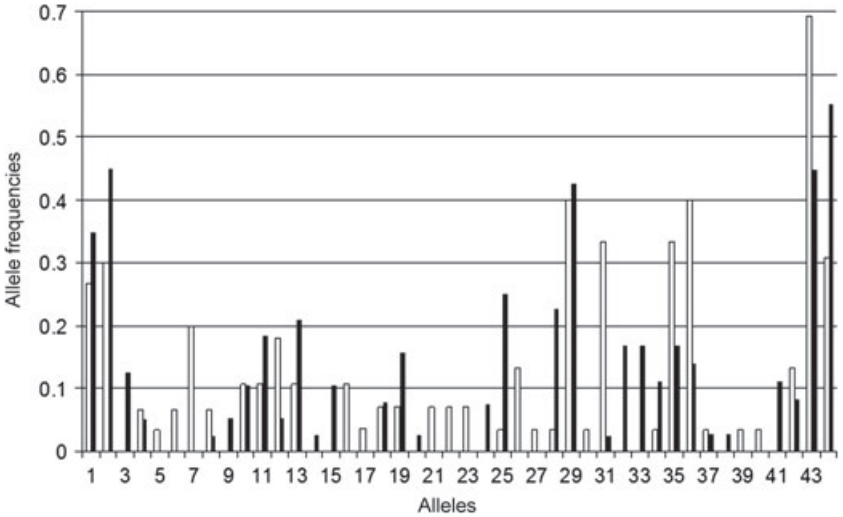

Fig. 1. Allele frequency shift for one microsatellite locus analysed in the butterfly *Lycaena helle* of one population (Massif Central, Mareuge, France) for the year 1991 (white) and 2006 (black). A clear shift in alleles and their frequencies was detected, whereas the total number of alleles remained similar (migration-mutation-drift equilibrium) (34 in the year 1991 and 31 in 2006). Data from Habel *et al.* (2011).

over a time frame of about 100 years. These observations are consistent with a population in migration-mutation-drift equilibrium. Populations experiencing such equilibrium lose alleles through drift at the same rate that migration and mutation introduce new alleles to the population. In general, such populations are considered to be fairly stable over the studied time frame (Piry, Luikart & Cornuet, 1999).

Likewise, the loss of genetic diversity does not always indicate a recent population bottleneck. A geographically restricted relict population of the red apollo butterfly (*Parnassius apollo*) in the Mosel valley of western Germany was completely monomorphic at six microsatellite loci (Habel *et al.*, 2009) that were polymorphic in French populations of the same species (Meglecz *et al.*, 2004). The genetic impoverishment of the Mosel valley populations was hypothesized to be the result of a severe population collapse during the 1960s as a result of indiscriminate insecticide spraying. However, this monomorphism turned out to older than the population collapse: samples collected before (1890–1960) and after (1960–today) both showed this lack of diversity at these microsatellite loci. This indicates that *P. apollo* was already genetically impoverished before the population collapsed. Similar population genetic stability despite small and isolated populations can be found in the endangered endemic Seychelles jellyfish tree, *Medusagyne oppositifolia*, which naturally occurs only on inselberg habitats (granitic outcrops). Here, despite fragmentation, the species was able (at least in its largest population) to maintain a high genetic diversity when comparing adult trees with progeny (Finger *et al.*, 2011).

In summary, we can delineate three different population genetic processes: (*i*) the loss of genetic diversity over time through genetic drift in isolated populations, (*ii*) migration-mutation-drift equilibrium, in which the loss of alleles is offset by the introduction of new alleles through migration and mutation, and (*iii*) the persistence of intraspecific diversity despite severe population bottlenecks as a consequence of long-term isolation.

The use of historical samples to detect, quantify and interpret potential effects of recent population bottlenecks, however, must be carried out with caution. Conclusions are only valid if historical sample sizes are representative (which is often not the case) and markers can be reliably genotyped. This is best highlighted by pointing out the consequences of a bottleneck on heterozygosity and allelic diversity. Heterozygosity is often quite insensitive to bottlenecks and even a population decline to two individuals will only lead to a loss of heterozygosity of $1/(2N_e) = 25\%$ in one generation (see Allendorf, 1986). By contrast, two individuals can only possess a maximum of four different alleles. This makes allelic diversity a better parameter for bottleneck detection. However, the effect of a bottleneck on allelic diversity depends on the total number of alleles found in a population and their frequencies, whereas the rate at which heterozygosity declines is always $1/(2N_e)$ regardless of the initial heterozygosity (Allendorf & Luikart, 2007). Accurate estimates of the number of alleles and their frequencies depend strongly on sample size, which is why a representative sampling of historical populations is critical.

V. THE RELEVANCE OF HABITAT HISTORIES – HABITAT PERSISTENCE *VERSUS* HABITAT TRANSFORMATION

Apart from population fluctuations due to environmental stochasticity and subsequent population bottlenecks, additional extrinsic forces play an important role in shaping the genetic composition of populations. The fragmentation

of formerly interconnected habitats typically increases the population structure within the species and fractures its genetic cohesiveness both of which often have a negative impact on the species (Zachos *et al.*, 2007; Sharma *et al.*, 2011). Some species, however, appear to be more tolerant to habitat fragmentation than others (e.g. Valqui, Hartl & Zachos, 2010). It is challenging to explain such contrasting responses to changes in habitat, which in turn makes it difficult to develop appropriate conservation strategies for species whose habitats are currently being destroyed. So far, only a few studies have explored the importance of population demographic histories in understanding a species' vulnerability to the negative consequences of habitat fragmentation (but see Leimu & Mutikainen, 2005; Angeloni, Ouborg & Leimu, 2011). Empirical studies using samples collected before and after fragmentation events for a variety of taxa are necessary to understand what affects the vulnerability of species to fragmentation.

Current research attempts to explain contrasting responses to changes in environmental conditions such as habitat fragmentation (Leimu *et al.*, 2006; Angeloni *et al.*, 2011; Finger *et al.*, 2012). For example, species that historically have existed in large, interconnected population networks may have been able to exchange genes among local habitat patches over short distances. Rapid and drastic environmental changes that disrupt these meta-populations may result in the sudden reduction or loss of gene flow leading to population differentiation. The loss of genetic diversity through increased drift and mating of related individuals may finally result in inbreeding depression. One example comes from tropical East Africa where forests with different habitat histories experienced different levels of habitat degradation (Habel & Zachos, 2012). The Chyulu Hills in southern Kenya are a naturally fragmented forest-meadow mosaic, while the neighbouring Taita Hills have suffered severe human-induced habitat destruction over the past few decades. The mountain white-eye (*Zosterops poliogaster*) inhabits both of these habitats, and genetic data from this species reflect the divergent habitat histories of these now similar habitat structures. The Chyulu Hills population, collected in 1938 and 2011, maintained its genetic diversity, and no genetic differentiation was detected in contemporary subpopulations. By contrast, the Taita Hills population sampled at different time points over the past 20 years shows a strong increase in genetic differentiation among local subpopulations (Habel *et al.*, in press). Together these findings demonstrate that fragmented habitat conditions (Chyulu Hills) do not necessarily have a negative impact on the intraspecific genetic diversity of a species *per se*, whereas fast transformations from interconnected to highly fragmented ecosystems (Taita Hills) may severely affect the biota living there. Such a sudden collapse of formerly intact habitat and metapopulation networks and the associated transition from (near-)panmixia to situations of reduced gene flow often have a negative impact on the maintenance of genetic variability and result in strong deviations from Hardy-Weinberg expectations and high inbreeding coefficients (Kadlec *et al.*, 2010; Konvicka, Benes & Schmitt, 2010). In the ground beetle *Carabus violaceus*, for example, recent habitat fragmentation due to road construction caused the split of a local population into two subgroups, resulting in strong genetic differentiation (Keller & Largiadèr, 2003; Keller, Excoffier & Largiadèr, 2005). The rapid development of genetically differentiated populations was also observed in the riverine cichlid fish, *Pseudocrenilabrus multicolor victoria*. Data suggests that this species experienced dramatic changes of its intraspecific structure across only a few years. While a clear isolation-by-distance pattern was detected in the first year,

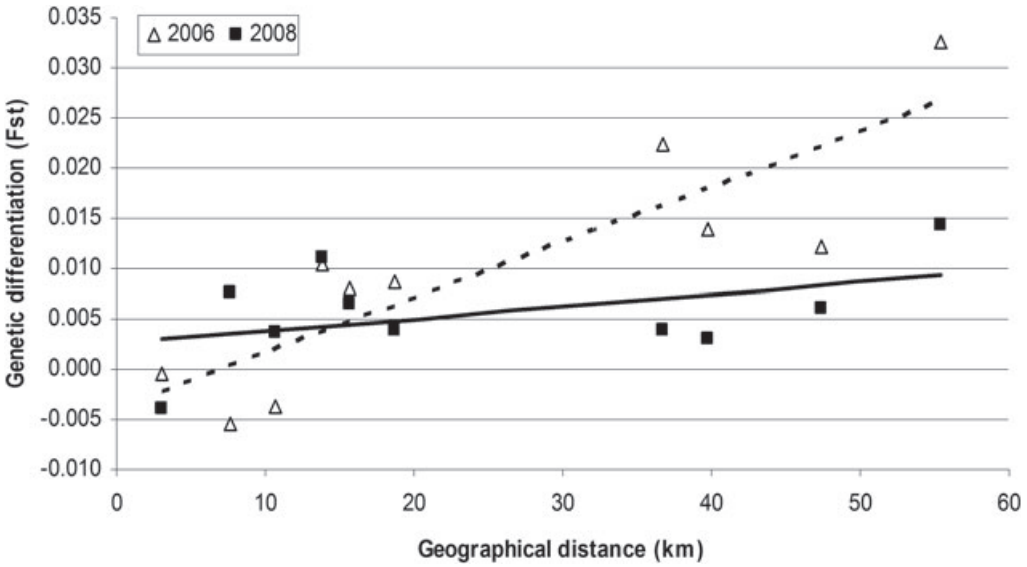

Fig. 2. An example of temporal instability in genetic structure. The same populations of the riverine cichlid *Pseudocrenilabrus multicolor victoriae* were sampled before (2006, triangles, broken line) and after (2008, squares, solid line) a flooding event. The original isolation-by-distance pattern found before the flooding event was eradicated after the event (From Crispo & Chapman, 2009).

the pattern was wiped out in the following sampling year. The authors suggested that this rapid change was the result of a severe flood between the sampling years (Crispo & Chapman, 2009) (Fig. 2). Rapid genetic responses were also found in large mammals with comparatively long generation intervals. Fickel *et al.* (2012) found that only 20 years (or roughly three generations) after the fall of the Iron Curtain, panmixia was re-established in red deer (*Cervus elaphus*) from the Bavarian-Bohemian forest ecosystem. In contrast to these examples, other studies indicate a genetic time lag for organisms living in changing habitat structures, as shown for the ground beetle *Carabus auronitens*, which today occurs in interconnected forest habitats, but still displays strong patterns of differentiation as a result of its past distribution pattern (Drees *et al.*, 2008).

The above examples highlight how the ecology of species and their demographic history affect their genetic structure. Owing to intrinsic species-specific requirements (such as microclimate or host specificity), some species may adapt to persisting in small and isolated populations and may have existed in such systems with low connectivity over long time periods. The species' historical distribution and population structure may have a substantial influence on its response to recent environmental changes (which may be less negative in generalist and genetically diverse taxa). Inbreeding depression, for example, may be lower in populations that have been small for a long time and consequently may have purged deleterious alleles, whereas a recent reduction in population size may cause stronger inbreeding depression (Lande & Schemske, 1985; Keller & Waller, 2002). Still, there seems to be controversy as to whether rare or widespread, and endemic or non-endemic species will be more prone to negative genetic consequences of habitat fragmentation (Habel & Schmitt, 2012; Habel & Zachos, 2012). Common species and large populations were found to be as, or even more, susceptible to the loss of genetic diversity through habitat fragmentation as rare species and small populations (Honnay & Jacquemyn, 2007; Angeloni *et al.*, 2011). It becomes apparent that in order to analyse the effects of environmental changes on populations, researchers have to consider both the past (e.g. pre-fragmentation, pre-bottleneck) and recent (e.g. post-fragmentation, post-bottleneck) population structure of the organism. Only analyses including multiple temporal samples of a population will be able to empirically disentangle recent rapid effects from past long-term processes.

VI. CONCLUSIONS

(1) Museums and other natural history collections as well as seed banks and similar natural sample deposits can provide material for temporal studies of genetic diversity and differentiation.

(2) Temporal studies should be planned based on the availability of historical material; contemporary sampling should be performed depending on historical sample availability.

(3) Co-dominant marker systems based on short fragments such as microsatellites and SNPs are most suitable.

(4) The future of conservation genetics will be based on genomic data provided from NGS. The combination of historical samples with these new technologies provides the most promising opportunities to study and protect biodiversity in the long term.

(5) Including historical samples allows for a more robust estimation of effective population sizes.

(6) Differing population histories may be key to understanding the potential persistence of species in fragmented environments.

(7) Time series have the potential to act as an important tool in gathering knowledge on the effects of historical events which may help us to understand modern biological and genetic processes and may further inform conservation management and aid decision-making.

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