YJBM

YALE JOURNAL OF BIOLOGY AND MEDICINE

CELEBRATING 90 YEARS 1928–2018

Conservation and Genetics

Charles B. Fenster[a,*], Jonathan D. Ballou[b], Michele R. Dudash[c], Mark D. B. Eldridge[d], Richard Frankham[e], Robert C. Lacy[f], Katherine Ralls[b], and Paul Sunnucks[g]

[a]Department of Biology and Microbiology, South Dakota State University, Brookings, SD; [b]Center for Conservation Genomics, Smithsonian Conservation Biology Institute, Washington, DC; [c]Department of Natural Resource Management, South Dakota State University, Brookings, SD; [d]Australian Museum Research Institute, Australian Museum, Sydney, NSW, Australia; [e]Macquarie University and Australian Museum, Sydney, NSW, Australia; [f]Chicago Zoological Society, Brookfield, IL; [g]Monash University, Clayton, Victoria, Australia

Humans are responsible for a cataclysm of species extinction that will change the world as we see it, and will adversely affect human health and wellbeing. We need to understand at individual and societal levels why species conservation is important. Accepting the premise that species have value, we need to next consider the mechanisms underlying species extinction and what we can do to reverse the process. One of the last stages of species extinction is the reduction of a species to a few populations of relatively few individuals, a scenario that leads invariably to inbreeding and its adverse consequences, inbreeding depression. Inbreeding depression can be so severe that populations become at risk of extinction not only because of the expression of harmful recessive alleles (alleles having no phenotypic effect when in the heterozygous condition, *e.g.*, Aa, where *a* is the recessive allele), but also because of their inability to respond genetically with sufficient speed to adapt to changing environmental conditions. However, new conservation approaches based on foundational quantitative and population genetic theory advocate for active genetic management of fragmented populations by facilitating gene movements between populations, *i.e.*, admixture, or genetic rescue. Why species conservation is critical, the genetic consequences of small population size that often lead to extinction, and possible solutions to the problem of small population size are discussed and presented.

INTRODUCTION

Why Conserve Species?

Students are drawn to biology because of the incredible diversity of life manifested at so many levels, from subcellular to organism, from physiology to behavior, from population to ecosystem. Much of this diversity represents genetic adaptations that allow for different life forms to grow and reproduce in a variety of environments and challenges. The mechanisms underlying the evolution of adaptations are well understood, and reflect the constant "filtering" action of natural selection acting on phenotypic variation among individuals, with at least some of this phenotypic diversity reflecting genetic variability [1,2]. With this process playing out over vast stretches of time and generations, we see the appearance

---

of multicellular organisms about 1.5 billion years after earth's origins, flowering plants from their non-flowering relatives about 140 million years ago (mya), primates from non-primates about 50 mya, hominids from the chimp lineage about 6 mya, and so forth [3]. Thus contemporary life reflects an uninterrupted sample of lineages, dating back to life's very origins. But now the process has become "broken" – in two senses. First, with the extinction of many species and populations within species, there are progressively fewer lineages to diversify and adapt to changing environments. Second, with the reduction of genetic diversity (and of species range and habitat diversity), each species will be able to adapt less quickly to the rapidly changing global environment. Thus, we are losing both much of the current incredible diversity of life and the mechanisms that promote new diversity at an alarmingly high rate (the loss of 50 percent of the world's vertebrate species since 1970) [4]. Despite increased dedication towards policy and management responses to the biodiversity crisis we are unable to reverse the extinction crisis [5].

Seeing the world of biological diversity through an adaptive lens helps us to appreciate the meaning of form and function among diverse organisms. What then of their conservation? What compelling case should be made for humans to spend effort and resources on species conservation? There are at least three arguments for species conservation having relevance especially for non-biologists: ethical concerns, sense of heritage, and human prosperity.

Ethical Concerns

Spiritual: A religious and non-scientific and certainly non-evolutionary perspective is that all organisms are created by a deity(ies), and human effort for their conservation thus serves a higher purpose. Following this thread to the Abrahamic religions, if humans are made in God's image, and God created organic diversity, then the mantle of responsibility falls to all of us to conserve all life forms (story of Noah, Book of Genesis, chapters 6-9). Early Christians also incorporated Judaic perspectives on the sanctity of life expressed through the teaching of Colossians 1:15-20: it is humanity's responsibility to preserve God's creation (Ps. 19.1; Gen.2.15) [6]. For other spiritual perspectives focusing on the unity of life, each species is a mutable expression of oneness, and species conservation is a way to maintain the holistic connection among all of life's forms. If we consider ourselves as morally responsible, then surely we are obliged to protect the diversity of organisms, especially given our role in their over-exploitation and demise.

Nonspiritual: Aldo Leopold encapsulated aesthetic and moral reasons to conserve biodiversity: "A thing is right when it tends to preserve the integrity, stability and beauty of the biotic community. It is wrong when it tends otherwise" (page 262) [7]. The ethical arguments focus on two questions, whether all living things are intrinsically valuable, and if so, whether having a varied group of intrinsically valuable objects is better than having a less varied group of intrinsically valuable objects [8]. A philosopher's answer might be that life is a good thing; consequently all living things are valuable. Thus, having more good things or more varied things is a positive attribute [8]. In sum, these spiritual and nonspiritual arguments underlie the UN World Charter for Nature 1982 [9], recognizing humankind as part of nature, where every life form is unique and warrants respect irrespective of its worth to human beings.

Sense of Place

A second and cultural argument, made by founders of the American environmental movement, is that our landscape has an indelible influence on how we see the world, as well as how we prosper on earth [7,10,11]. Alas, this perspective is largely lost on most Americans, although it can be found in many other areas of the world. Just as many Americans might identify with "mom," "apple pie," and "baseball," even more so does the cultural, physical *and* biological landscape affect each of us individually and as communities. New Yorkers (NYC) recognize the rivers, skyscrapers, Central Park, corner deli, etc., as part of their daily fare whose presence resonates pride in place, and their absence, whether through personal migration or development, registers loss. The view looking northwards up the Hudson River Valley from the Cloisters Museum is unparalleled in evoking a pastoral sense of being, while sunset reflected on the red brick-faced apartment buildings may trigger the same sense of being as the transcendent tranquility of sunset in canyon or butte country. Similar analogies can be made by those who inhabit this sweeping country of ours, from amber waves of grain to the towering redwoods and from sea to shining sea. Lost in all of this is our recognition, as Americans, of the importance of species diversity. Shouldn't we be comforted by the sight and sounds of native prairie, monarch butterflies, roaming buffalo, howling wolves, crying loons, the flaming fall colors of the eastern deciduous forest, the annual geese migrations, in total, native plants and animals, and members of other kingdoms? Or are we content to sit back, see the extinction of our native organisms, and their communities, some of which date back tens of millions of years (*e.g.*, grasslands, eastern deciduous forests), only to be replaced by invasive foreign species whose presence also incurs considerable economic impact. Invasive microbes, animals, and plants cost the US economy 120 billion dollars annually and it is

only getting worse [12]. In short, pride of being and pride of place should reflect recognition of native species as an integral part of our culture [13].

Human Prosperity

A third and increasingly persuasive argument is that human prosperity hinges on biological diversity for two essential and related reasons, both representing an ecocentric perspective [7]. First is the recognition that humans, as animals, live and attempt to prosper within an environmental context. Consequently, what is good for the environment should in general be good for humans. For example, predators such as mountain lions and wolves might make us feel unsafe with reason, but if they in turn control deer populations that harbor tick-bearing Lyme disease, then they provide an essential service associated with disease control [14]. Another example is that biological diversity in Finland is positively associated with microbial diversity that in turn has a positive effect on human health as manifested by fewer allergic responses [15]. Similarly, environmental mitigation that results in pollinator health or improved water quality will have consequences on the amount and quality of the food we eat and the water we drink. This argument is embodied within the concept of ecosystem services, or ecosystem goods, the benefits provided by ecosystems to humans [16].

Ecosystem services are a value-based approach that makes explicit our implicit actions. When a community allows a developer to drain wetlands for housing, the community recognizes housing as having greater value than the wetlands. Unfortunately this is exactly the choice that was made in Louisiana, even though the Louisiana Coastal Wetland Conservation and Restoration Task Force in 1998 emphasized the danger of wetland destruction of the type that contributed to the destructive force of Hurricane Katrina in 2005 [17]. Thus, the ecosystem services perspective draws attention to both the short and long-term value to humans of the conservation or preservation of ecosystems. Rough estimates of the value of ecosystem services are that they are at least equal to the total value of the world's economy [18]. Intact ecosystems, and thus the services they provide, rely on the species and the interactions among species found in that ecosystem, which brings us back to the role of adaptations. Adaptations allow species to exist where they are found and ultimately ecosystem function relies on the individual species and the traits they manifest in that ecosystem [19,20]. In short, biodiversity is the foundation for intact ecosystems [21], and the loss of species may very well lead to the end of civilization as we know it [22]. For further information see ActionBioscience.org, a non-commercial, educational website created to promote bioscience literacy (active since 2000) [23]. The site provides links to articles discussing the benefits provided by life's diversity and insights into new advances in biodiversity research.

Important Questions

We have established that a *raison d'être* for species conservation reflects adaptive traits allowing a species to survive in a particular environment *and* therefore contribute to ecosystem functioning. We turn now to population size, a surprisingly important modulator of the adaptive evolution we hope to conserve. Furthermore, population size can be an important determinant of the frequency of harmful alleles (alleles are different forms of a gene), *i.e.*, the expression of unfavorable alleles with consequent damage to organisms' health. This, in turn, can contribute to population and species extinction. The questions addressed are: 1) Why is population size so important? 2) How is population size related to species conservation? and 3) What can we do to modify population size for species remediation?

POPULATION SIZE

Intuitively, one expects as the number of individuals in a population gets smaller then random events might lead to a greater probability of extinction than for a larger population. Population size, as it surprisingly turns out, also mediates the role of selection and the accumulation of harmful alleles, having considerable consequence for evolution and conservation.

Effective Population Size (N_e)

The genotypes of the next generation are a sample of gametes from the parental generation that result in the progeny. To understand the implications of the sampling process let's embark on an exercise with an idealized situation of one gene, *e.g.*, the "A" gene, variable in the population, with two alternative forms (alleles), A1 and A2, with equal frequency (*i.e.*, 0.5 each). Homozygote genotypes (A1A1, A2A2) produce only A1 or A2 gametes, respectively, while the heterozygote genotypes (A1A2), produce gametes (reproductive cells) carrying either allele with equal frequency according to Mendel's first law of segregation. If mating between genotypes is random, then a zygote (fertilized egg that can develop into a new individual) is formed from the random fusion of gametes, two A1 gametes fusing results in an A1A1 zygote, and so forth. Taking two coin tosses as an analogy for the fusion of two gametes, each carrying one copy of a gene, we expect trials of two tosses would result in either Heads (Head, Head), Tails (Tails, Tails) or Heads and Tails (Head followed by Tails or Tails followed by Heads).

Thus with a fair coin, we expect coins tossed two at a time, many times, would agree with the prediction of a binomial sampling event (Box 1) (= in genetic terminology, Hardy-Weinberg equilibrium based on expectations from binomial sampling theory, see any genetics textbook), that 1/4 of the tosses would be head-head, 1/4 tail-tail, and 1/2 head-tail or tail-head. However, it should also not be surprising that if we conduct only a limited number of coin tosses, for example 10, representing the fusion of five pairs of gametes to produce five individual zygotes, we might observe deviation from the expected, with more of one type and therefore less of the other type. That is, if a population of five individuals has an equal proportion of A1 and A2 alleles (the population has 5 A1 alleles and 5 A2 alleles), then the next generation of five individuals might very well have six of one and four of the other, and so forth. The binomial sampling distribution results in a relatively large change in the frequency of either allele through chance alone. The variation introduced by sampling of gametes is known as genetic drift, and following well understood binomial probability theory (Box 1), the variation from expected increases with smaller sample sizes [24].

Natural populations never behave as simply as in the above example in three important ways, although there are other considerations that we leave for a specialist audience. First, population sizes are almost never constant across generations. Consider fluctuations in the size of the human population throughout the last 100,000 years [25]. At the time that *Homo sapiens* (humans) began their migration out of Africa, some 70,000 years ago, the world's human population consisted of tens of thousands. By the dawn of agriculture 10,000 years ago, human population size is estimated to be about 4-5 million, and first reaching one billion at the time of the Napoleonic wars 200 years ago. But the increase has not been steady. For example, the various plague epidemics that swept through Europe for 500 years from the years 1000-1500 greatly reduced population sizes with estimates of from 1/3 to 1/2, depending on the plague episode and region. There are still former agricultural regions of Sweden that were abandoned in the later plagues and have been left to forest. China's population was reduced by one-half in the thirteenth century by a combination of plagues, invasion and political unrest, etc. The lesson imparted to our understanding of human population size across human history is that the likelihood of allele frequencies changing randomly over time depends not so much on just how many individuals there are in the previous generation but also how many progeny there are in the next generation, a.k.a, the effective population size. Thus, population geneticists derived the concept of the effective population size (N_e) or the population size of individuals corresponding to the observed change and fluctuation of allele frequencies due to drift [26,27]. A population size of many thousands giving rise to only five zygotes in the next generation (our example), will experience a contribution of chance to allele frequency change representative of a

---

Box 1. Glossary of terms.

Binomial sampling distribution: This is the probability distribution of the number of samples that have either one of two outcomes, such as, heads/tails, success/failure, yes/no, allele state one/allele state two, and so forth. There are two parameters, n, the number of independent experiments and p, the probability of one of the two outcomes (*e.g.*, success, tails, a gamete carrying one or the other allele). Thus, using the binomial distribution we can model the expected distribution of the number of heads for a US coin for any number of trials. For example, if we flip a fair coin 10 times, with a mean and peak frequency of 5, there will be some samples of 10 flips having as few as 0 heads and some with all, or 10 heads. Similarly, we can model the expected distribution of the allele frequencies the following generation given a sample size of n number of progeny (each progeny representing an independent sample of two gametes).

Harmonic mean: The harmonic is one of many types of means (the average being another) and it typically gives the smallest mean because small numbers are weighted more heavily in its calculation. It is the average of the reciprocal of the reciprocals. For example, consider 10 numbers, 10, 100, 1000, 10,000, 100,000, 1,000,000, 10,000,000, 100,000,000, 1,000,000,000 and 10,000,000,000 (10 to 10 billion in US terms), then their harmonic mean average is the reciprocal of the mean of their reciprocals: $[1/10 (0.1, 0.01, 0.001, 0.0001, 0.00001, ..., 0.0000000001)]^{-1}$ equals $[1/10 (0.1111111111)]^{-1}$ equals $(0.0111111111)^{-1}$ equals 90 (approximately). The harmonic mean is used to calculate the size of a population across generations to determine the opportunity for inbreeding and drift to occur. Thus, very small populations have a bottleneck effect on subsequent genetic variation, as the small number of individuals contribute disproportionally to future levels of genetic variation within the population. These bottleneck effects can be mitigated by crossing with other populations, *i.e.*, genetic rescue.

Poisson distribution: This is a probability distribution of the number of events that happen in a discrete space or time, and for our purposes, the number of progeny produced by families. The mean and variance are equal to one another in a Poisson distribution. Population genetic theory demonstrates that when the variance of progeny size among families exceeds the expected based on a Poisson (*e.g.*, zero population growth, mean family size = 2, variance of family size > 2), then the rate of increase of inbreeding and loss of genetic variation exceeds the expectation based on population size. That is, some families are contributing disproportionally to the next generation. In contrast if the variance of family size is less than the mean in the same scenario, then the rate of increase of inbreeding and loss of genetic variation is less than expected. Typically, zoo management schemes attempt to minimize variance in reproduction among males and females used in the breeding program, so as to increase the effective population number.

population of five, not thousands, and consequently will have an effective population size closer to five.

Population genetic theory provides an answer to how important sampling error, genetic drift, will be to a population with fluctuating populations: it depends on the harmonic mean population size across generations (Box 1) [24]. The harmonic mean is dominated by small numbers, so a population that grows from 10 to 100 to 1000 to 10,000 to 100,000 to 1,000,000 to 10,000,000 to 100,000,000 to 1,000,000,000 million to 10,000,000,000 or from 10 individuals in generation 1 to 10 billion individuals in generation 10, *i.e.* with exponential growth rate of 10 fold, will act more like a population of 90 individuals over the 10 generations, in terms of the role of sampling error, than the later larger populations sizes. Once genetic drift occurs, as it does in the earlier generations of this example, its effects do not automatically reverse to the former state. That populations may act different genetically in terms of the role of random fluctuations due to drift than what one might expect based on the census number of the population has surprisingly profound implications on the origins and conservation of biodiversity.

Two other factors reduce the effective population size relative to the observed census size, and thus suggest that drift has an even more important role in the transmission of alleles across generations: variation in the sex ratio and variation in reproductive success among families. For many animals, and to some extent humans, variation in reproductive success is much less in females than males. That is, some males have enormous reproductive success relative to others that may never mate. The reason is easy to see when one considers polygamy. In species where males have harems of females, and there is a one-to-one sex ratio of males to females at birth, then the population in the next generation will more likely reflect the sample of males that mated than the total population of males, some of which never mated. A striking human example is that approximately 10 percent of all Central Asian males carry a Y-chromosome so similar that it could only have been inherited from one or a small group of related males, likely descendants of Genghis Khan, his brother, and immediate male descendants who kept large harems [28]. Likewise there are a number of other men who lived in central Asia that left a huge number of descendants [29]. The final factor is very large variation in family size. Some male-female mating pairs may have enormous family size whereas others may have no offspring, and if this variation is greater than a variance predicted by a Poisson distribution (Box 1) [30] then the effective population size will be less than the census size. Genetic diversity is depleted because a large portion of the genes in the subsequent generations came from the few highly successful families.

Consequences of Genetic Drift

These three factors, fluctuation of population size, effectively unequal sex ratios and large variance of reproductive success across mating pairs all result in populations acting effectively, in terms of the role of drift, as if they were much smaller than the census size. Indeed, a rough rule of thumb is that the long-term effective size of a wildlife population is roughly 11 to 14 percent of the census size [31-33]. The ratio varies widely based on life history, especially age at sexual maturity and adult lifespan [34]. Species with very high fecundity (fish, oysters, shrimp, and seaweed) have much lower ratios of about 10^{-3} to 10^{-6}.

Now we investigate the consequences of effective population size and drift in terms of our understanding of the origins and maintenance of biodiversity. We will explore how drift may increase the frequency of harmful alleles and contribute to population extinction. But first we need to briefly discuss fitness and how it relates to the increased frequency of alleles that increase fitness relative to those alleles that decrease the fitness of the individuals that carry them.

Fitness is the performance of individuals relative to other individuals in the population, where performance is the propensity to produce offspring, thus a combination of survivorship to reproduction with reproductive success (both the quality and quantity of offspring) [24]. When fitness variation is associated with genetic variation, or variation in trait expression contributes to fitness variation and this trait variation has at least some genetic origins (heritable), then the expression of the optimal trait will increase in the population [1,2]. For example, human height variation is explained by both the alleles affecting height inherited from one's parents and one's nutritional status prior to maturity [35]. Thus, if selection favors individuals of greater height, *e.g.*, taller male graduates of the US Naval Academy have more offspring, while shorter stature appears to be favored in the island of Sardinia, then height will evolve in these populations, increasing in the USA while decreasing in Sardinia [36,37]. The fitness variation for male graduates of the US Naval Academy appears to be related to taller men being more likely to have second families with younger second wives while shorter stature on Sardinia is consistent with mammals larger than rabbits evolving to be smaller on islands due to reduced resource availability. Fitness is abbreviated as *w*, such that the fitness of one genotype relative to another is either lower or higher. It may help to understand fitness if you consider that *w* can be viewed as a weighting factor, the higher the fitness or *w* of a genotype, the more likely those who express the optimum trait, or carry that favored genotype, will survive and reproduce.

The cause underlying fitness variation is any number of features of the environment, either biotic or abiotic or

both, resulting in selective pressures, the action of which is natural selection (any evolutionary biology textbook). The amount of selection acting on a trait or genotype is abbreviated as "*s*" and is related to fitness as $w = 1 \pm s$. If an individual because of its phenotype has lower fitness, then *s* is acting against this phenotype, and vice versa. An important consideration is that in addition to selection varying in sign, favoring or disfavoring a genotype, it can also vary in magnitude, varying from very weak to very strong. When this process is extrapolated for many generations (sufficient time), the trait, or a particular level of expression of the trait, that is favored by selection will become a fixed feature of the species (analogously consider selection imposed by humans resulting in the many dog breeds).

Drift, Harmful Alleles, and the Role of Selection

It may seem non-intuitive, but sometimes alleles that decrease fitness can increase in frequency, despite selection acting against them. Although an allele may decrease the performance of an individual, that allele may nevertheless increase in frequency. How so? The answer lies in the balance between selection favoring a specific genotype, and the effective size of the population (N_e), which determines the importance of drift. This will become clearer when we next return to our example of number of coin flips as analogous to the sampling of gametes carrying particular alleles, A1, A2.

Consider the following coin toss experiment. We expect to observe an equal frequency of Heads and Tails, but as before, with a limited number of tosses it would not be surprising if we observed say a 6:4 ratio of Heads:Tails with a sample size of 10 tosses, that is, Heads are 1.5 times as common as Tails. However, with a much larger sample of tosses, say one million, we would be very surprised to observe such a large deviation from a 50:50 ratio. Now let's use a weighted coin, favoring Heads over Tails. Remember that fitness is symbolized by *w*, or a weighting term. Thus a weighted coin is analogous to one allele (one side of the coin) favored, the alternative allele disfavored (the other side of the coin). If the coin is weighted heavily enough, say Heads is favored, then Heads always appears. This is analogous to very strong selection favoring one allele over another. No matter how many tosses, Heads predominates in the sample. Now consider a much more subtle weighting, perhaps favoring Heads 51 percent of the time, analogous to a slight fitness advantage of the A1 allele over the A2 allele. With a sample of a million tosses we would expect close to 510,000 Heads and a corresponding 490,000 Tails. We would be very surprised if Heads did not at least surpass Tails in absolute number in this sample of one million tosses. However, with a small sample of tosses, even with the slight favor of Heads over Tails, we would not expect

to see Heads predominate all the time. For example, binomial sampling theory tells us that in a series of trials where the coin is flipped 10 times, and repeating the trials of 10 tosses 100 times, then 35 percent of the time, or 35/100 trials, Tails would appear six or more times, even though Heads is slightly favored. Equating this to populations and allele frequency changes, even harmful alleles can increase in frequency if population sizes are small enough to facilitate drift.

The theory behind this phenomenon focuses on the product of effective population size and selection [38]. If either selection acting on an allele is weak ($s \ll 1$), or the population is small (N_e very small, *e.g.*, <100), or a combination of the two, then the frequency of the harmful allele will reflect more the action of drift and less the action of selection. Formally, if the absolute value of the selection coefficient, either acting against the allele (*i.e.*, harmful) or favoring the allele (*i.e.*, beneficial) is smaller than the reciprocal of twice the effective population size, *i.e.*, $s < 1/2N_e$, the dynamics of the allele will be determined predominantly by drift [39]. However, the problem for a population is worse than simply harmful alleles increasing in frequency. In fact, populations will adapt evolutionary (a change in phenotype across generations reflecting genetic changes) more slowly the smaller the N_e. This is not only theoretically derived, but also empirically observed. For example, artificial fruit fly populations can evolve to adapt to ethanol poisoning, but smaller populations evolve at a lower rate of change than larger populations [40,41]. The genetic mechanism for slower rates of evolution in smaller populations is that beneficial alleles are often lost by drift and deleterious alleles increase in frequency, also by drift. In other words, selection becomes a less powerful filter the smaller the population.

Conservation Consequences of Drift

We have established that drift, the sampling of a limited number of gametes represented in the next generation zygotes, is a phenomenon of all populations and drift becomes a large force for allele frequency change as populations become smaller. There are two important consequences of drift for species conservation: drift can cause even harmful alleles to rise in frequency by chance alone, especially in small populations, and smaller populations are less likely to evolve adaptively to new environmental challenges.

THE CHALLENGE FOR CONSERVATION

Threatened and endangered species are recognized as species with a limited number of populations consisting of a limited number of individuals. About five percent of all known species, 91,000 of 1.9 million, are on the

International Union of Concerned Scientists for Nature (IUCN) Red List of Threatened Species [42]. However, it is even worse than this because many species have not been described (catalogued by science). Further, even if a species is known, the information about its status is often so limited that is it listed as "data deficient." Additionally, even if the species is known and there is enough information, the IUCN listing process takes so long that there is a considerable time lag between when experts know a species is in trouble and the time it actually gets properly listed.

The crisis is that the number of species threatened with extinction is approaching 1000 times the normal rate throughout evolutionary time and all of this increase is due to human activities. We see the contribution of humans to biodiversity loss through many temporal and spatial axes. Since hominids evolved and migrated out of Africa, the world's large land mammals have often been hunted to extinction, with some experts estimating that the largest mammals to be found outside of zoos in the near future will be cows [43]. We are now observing the world-wide massive decline of numbers of individuals within species as diverse as insects, reptiles and birds [44-46]. There is an overall cumulative decline of biological diversity across the planet [47]. Thus, even species that are currently common may soon experience the spiral towards extinction. The ability of organisms to move, migrate seasonally, or follow environmental gradients is also rapidly declining as a result of fragmentation of the landscape through human influence [48,49]. Populations that are limited in number, contain few individuals, and have decreasing likelihood of movement between populations, all contribute to a scenario of drift playing an increasingly large role in the evolution of most remaining species. In addition, with human caused climate change, the ability of species to adapt in place will be limited by drift. Populations will experience inbreeding decline through homozygosity for harmful alleles, and will have difficulty finding favorable environments in an environment fraught with human barriers.

Action

To reverse the consequences of small populations accumulating harmful genetic variants and having lower ability to evolve in response to environmental selective pressures, a number of vocal biologists have proposed that genetic rescue, the movement of individuals between populations, should be the default practice of conservation managers [49-51]. It is an amazingly simple, yet intuitive approach. No human society promotes mating within the nuclear family (*e.g.*, father-daughter, sister-brother, etc.), and about the closest degree of relationship allowed is first cousins (Leviticus 18) or second cousins (*e.g.*, Catholic doctrine). Most eukaryotes (organisms comprised of

Figure 1. **King Charles II**. This remarkably unflattering portrait of King Charles II (despite the artist's attempts to do otherwise) demonstrates the harmful consequences of close inbreeding in humans and the vast majority of non-haploid organisms. Because of prior generations of mating with close relatives, Charles II manifested the inbreeding slightly higher than expected in the offspring of a brother-sister mating (see text) [56]. KHM-Museumsverband.

nucleated cells) also avoid close inbreeding by various mechanisms, *e.g.*, mammalian males often disperse from the natal unit (prairie dogs, chimpanzees), some fungi and plants have incompatibility alleles that prevent them mating with themselves or with close relatives, and many flowering plants have remarkable floral features promoting mating between individuals while preventing mating with themselves (a.k.a., outcrossing) [52]. The nearly universal avoidance of inbreeding represents protection against harmful recessive alleles that inevitably exist in populations [53,54]. An interesting aside is that some animals and plants do actively inbreed, *e.g.*, naked mole rat, many soy bean cultivars, mostly as a response to limited mate availability, or in humans, preserving the "purity" of a royal lineages such as the Hapsburg dynasty. The frequency of these harmful alleles reflects the balance between the opposing forces of mutation and selection. Mutation introduces the harmful alleles and selection can remove them when they are in the homozygous form [55]. Harmful alleles are often not fully recessive, causing mild reduction in fitness in heterozygotes, and can rise to relatively high frequency through drift [30]. Re-
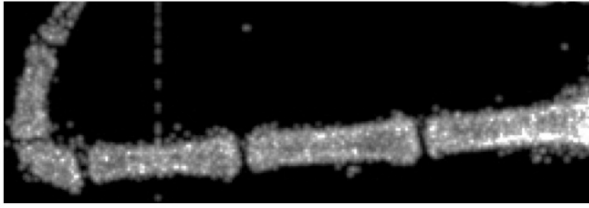
Figure 2. The Florida Panther was reduced to a few individuals and demonstrated typical signs of inbreeding, including a high frequency of sterile sperm, and, in the case of cats, a high frequency of kinked tails, shown in the figure. The Florida Panthers also had low genetic variation, typical of an inbred population. Female panthers from Texas were introduced to the inbred Florida population, and the phenotypic manifestations of inbreeding disappeared simultaneous with the increased vigor of the population (see text) [60]. Reprinted from Current Biology, 3, M. E. Roelke, J. Martenson and S. J. O'Brien, The consequences of demographic reduction and genetic depletion in the endangered Florida panther, 340-350, 1993, with permission from Elsevier.

lated individuals are more likely to share harmful alleles, inherited from common ancestry, than are unrelated individuals. Thus, avoiding mating with relatives results in progeny that are not debilitated by inheriting two copies of the same harmful allele, whether fully recessive or not. There are many human examples demonstrating the negative consequences of inbreeding, but none is perhaps so interesting as the case of the last Spanish Hapsburg King, Charles II (Figure 1). Because of the proclivity of the royalty to marry relatives, the level of homozygosity of Charles II was greater than one would expect following a brother-sister mating [56]. Charles II manifested many of the conditions associated with inbreeding depression, poor overall performance, reflected in such characteristics as the inability to keep his tongue in his mouth, erectile dysfunction, sterility, and lower intelligence. Without an heir, the succession was open, leading to the War of the Spanish Succession (1701-1714), having repercussions for Europe over the short and long-term.

When populations become inbred, then the obvious solution is to facilitate crossing between one inbred population with another population, where the individuals are less related to the former. This notion of crossing unrelated individuals resulting in offspring of higher performance underlies the green revolution where highly productive crops are often heterozygous, expressing superior performance due to the absence of homozygous harmful alleles. This phenomenon is known as hybrid vigor or heterosis [57,58]. Concordant with the above explanation for avoiding mating with relatives, heterosis is mostly associated with a dominant-recessive relationship between the allele favored by selection and the deleterious reces-

sive allele [59]. Thus, matings between two unrelated individuals leads to progeny having higher performance than inbred progeny. Two examples demonstrate the challenges and benefits of genetic rescue from a conservation perspective.

First consider the case of the Florida panther [60]. The Florida panther is a race of the new world cougar and was formerly found throughout the southeastern USA. However, by the 1990s the population was very small and restricted to the southern end of Florida, and the individuals manifested inbreeding depression as low sperm counts, kinked tails, and a population in decline (Figure 2). The transport of eight panthers from Texas, genetically closest to the Florida race, led to the immediate reversal of population decline. Although still closely monitored, the Florida panther is in much better health than before genetic rescue. Second are a number of cases involving hermaphroditic flowering plants having self-incompatibility ensuring an outcrossing mating system. Self-incompatible mating systems ensure that pollen cannot fertilize the eggs of the same plant. These systems are based mostly on one or two incompatibility loci, where the female reproductive part accepts only pollen that does not share any alleles at these loci [61]. Strong selection maintains incompatibility alleles in populations, since genotypes with rare alleles enjoy a mating advantage (they are more likely to mate with others) [62,63]. However, with fragmentation of populations and reduced population size, these incompatibility alleles can be lost from the population by drift, despite selection acting on their maintenance and this has happened in a number of instances for species of conservation concern. For example, the Lakeside daisy (Figure 3) is restricted to dry prairie, with thin soils overlying limestone and requires open sites and full sun. Unfortunately, fire suppression and limestone quarrying destroy the plant's habitat. While once widespread in Illinois, by the late 1970s the daisy was reduced to one population, and with the quarry equipment closing in, an amateur collector saved the last four plants. Unable to propagate the plants by seed, a conservation biologist determined that the species is self-incompatible, and the four Illinois specimens were all of the same mating type, thus unable to reproduce with one another [64]. After some debate concerning purity issues, the Illinois plants were successfully crossed with plants from a large Ohio population, and the seedlings were successfully established in several sites in Illinois (Figure 3) [64]. Crosses between populations were also responsible for the successful implantation of the recovery plan for the Florida *Ziziphus*, a plant with similar biological conditions (self-incompatible angiosperm species with each population, except one, comprising a single clone, with different clones in different populations) [65].

While the above examples simply demonstrate how

Figure 3. In Illinois, the last remnant population of Lakeside daisy was reduced to four individuals that unfortunately (by chance) all shared the same incompatibility genotype and consequently were unable to mate (make seed) with one another. The investigator [64] recognized that the species is self-incompatible and was able to produce copious seed by mating the Illinois individuals to individuals from Ohio of different incompatibility genotypes. Seed was germinated and in the early 1990s, seedlings were transferred to two sites in Illinois having the physical and biotic features of typical Lakeside daisy habitat. The two populations are still thriving. Photo courtesy of Juanita Armstrong-Ullberg and the Forest Preserve District of Will County, Illinois.

genetic rescue can restore a population's vigor, there are several issues to consider that are associated with outbreeding depression. Outbreeding depression is the condition where the progeny from a cross between individuals from different populations have lower performance or fitness than "pure" individuals that are progeny of two individuals from the same population. The first issue is that some argue that genetic rescue will disrupt the purity of a population or species. However, without genetic rescue, preservation of a population's purity may lead to extinction. Also, it is likely that mixing of populations has been a natural phenomenon throughout the history of species, as ranges of races or populations once separate come into contact once again through range expansion following favorable environmental change [66]. Thus, the supposedly "pure" local populations were not originally genetically invariant, but instead were dynamic entities with a constantly adapting array of genes that periodically got refreshed through exchange with nearby populations or even other species.

Yet there are potential negative consequences (outbreeding depression) of population mixture reflecting two genetic sources. If the populations have different chromosome content, as is sometimes the case with plant species having diploid and polyploid populations, then mixing populations can lead to hybrid sterility [49]. Furthermore, if populations are adapted to different environments, then mixing populations may lead to a decrease

of fitness of the hybrids relative to the parents [67]. Even so, selection is a powerful enough force that the hybrid population may evolve high fitness [68]. However, many small populations are not well adapted to their current environment due to the accumulation of harmful alleles and rapidly changing environmental conditions due to climate change. It is important to remember that inbreeding as a result of small population size, and the consequent expression of inbreeding depression is a universal phenomenon (for organisms that normally mate with other individuals of the same species), while outbreeding depression is much rarer and its occurrence can be predicted [69]. Practical guidelines for avoiding outbreeding depression during genetic rescue are described in [49,50].

A lack of understanding of the importance of population size, genetic variation, and genetic exchange among populations can result in policies that do not protect the diversity of life around us and can even accelerate the tragic losses. For example, the recent Recovery Plan for the Mexican Wolf, a subspecies of the gray wolf, proposes that management will promote growth of the small population that was reestablished from released captive wolves, but only up to the size that was estimated to be just adequate to allow short-term persistence [70]. Above that minimal size, any supposedly "excess" wolves would be removed, and no wolves would be allowed to venture north where they could mate with northern subspecies of gray wolves. Thus, the "recovery" plan provides little prospect for the restoration of a healthy and naturally evolving population of Mexican wolves. Genetic rescue addressing the consequences of small population size is too rarely employed [49-51].

CONCLUSION

Arguments for species conservation are presented. The population genetic theory and consequences of small population size are described. The cataclysm of species extinction necessarily reflects a progression of steps including small population size, inbreeding, loss of ability to adapt, and final extinction. A default strategy for species conservation must include consideration of genetic rescue: admixture of individuals between populations to restore population genetic vigor [49,50]. If it is not possible to give organisms the opportunity to move across the landscape, crossing barriers such as roads or dams, then we must move individuals between populations [71]. The harmful consequences of drift reflect sampling processes. Unfortunately we have only one earth, *our earth*, and thus little time to conduct experiments on how best to conserve biodiversity at the planet level. We as individuals, and collectively as a society must take on the mantle of species conservation based on scientific principles.

REFERENCES

1. Darwin C, Wallace A. On the Tendency of Species to form Varieties; and on the Perpetuation of Varieties and Species by Natural Means of Selection. Zool J Linn Soc. 1858;3:45–62.

2. Darwin C. The Origin of the Species. London: John Murray; 1859. p. 502.

3. Freeman S, Herron JC. Evolutionary Analysis. 5th ed. Boston: Pearson; 2014. p. 850.

4. WWF. The Living Planet Index. [Internet]. cited 2018. June 15. Available from: http://www.livingplanetindex.org/projects?main_page_project=LivingPlanetReport&home_flag=1

5. Tittensor DP, Walpole M, Hill SL, Boyce DG, Britten GL, Butchart SH, et al. A mid-term analysis of progress toward international biodiversity targets. Science. 2014;346:241–241.

6. CRI. Christians and the Environment: How should Christians think about the environment? [Internet]. cited 2018. June 15. Available from: http://www.equip.org/article/christians-and-the-environment-how-should-christians-think-about-the-environment/

7. Leopold A. A Sand County Almanac, and Sketches Here and There. New York: Oxford University Press; 1949. p. 240.

8. Brennan A, Lo YS. Environmental Ethics IN Zalta EN, editor. *The Stanford Encyclopedia of Philosophy*. Metaphysics Research Lab, Stanford University: Winter 2016 Edition.

9. United Nations World Charter for Nature. Environ Conserv. 1983;10:67–8.

10. Thoreau HD. Walden; or, Life in the Woods. Boston: Ticknor and Fields; 1854. p. 359.

11. Carson RL. Silent Spring. Boston: Houghton Mifflin; 1962. p. 368.

12. Pimentel D, Zuniga R, Morison D. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ. 2005;52:273–88.

13. Chatwin B. The Songlines. London: Jonathan Cape; 1987. p. 293.

14. Telford SR. Deer reduction is a cornerstone of integrated deer tick management. J Integr Pest Manag. 2017;8:10–1093.

15. Hanski I, von Hertzen L, Fyhrquist N, Koskinen K, Torppa K, Laatkainen T, et al. Environmental biodiversity, human microbiota, and allergy are interrelated. Proc Natl Acad Sci USA. 2012;109:8334–9.

16. Costanza R, d'Argre R, de Groot R, Farber S, Grasso M, Hannon B, et al. The value of the world's ecosystem services and natural capital. Nature. 1997;387:253–60.

17. Louisiana Coastal Wetlands Conservation and Restoration Task Force and the Wetlands Conservation and Restoration Authority. Coast 2050: Toward a Sustainable Coastal Louisiana. Baton Rouge (La.): Louisiana Department of Natural Resources; 1998. p. 161.

18. Costanza R, de Groot R, Braat L, Kubiszewski I, Frioramonti L, Sutton P, et al. Twenty years of ecosystem services: how far have we come and how far do we still need to go. Ecosyst Serv. 2017;28:1–16.

19. Gleason HA. The individualistic concept of the plant association. Bull Torrey Bot Club. 1926;53:7–26.

20. Antonovics J. Plant population biology at the crossroads. Input from population genetics. Syst Bot. 1976;l:234–45.

21. Cardinale BJ, Emmett Duffy J, Gonzalez A, Hooper DU, Perrings C, Venail P, et al. Biodiversity loss and its impact on humanity. Nature. 2012;486:59–67.

22. Ehrlich PR, Ehrlich AH. Can a collapse of global civilization be avoided? Proc Biol Sci. 2013;280:20122845.

23. Actionbioscience: Biodiversity: Why Should We Work to Preserve the Variety of Species? [Internet]. cited 2018. June 15. Available from: http://www.actionbioscience.org/biodiversity/

24. Crow JF, Kimura M. An Introduction to Population Genetics Theory. Minneapolis: Burgess Publishing Company; 1970. p. 591.

25. Wikipedia. org: World population. [Internet]. cited 2018. June 15. Available from: https://en.wikipedia.org/wiki/World_population

26. Wright S. Evolution in Mendelian populations. Genetics. 1931;15:97–159.

27. Wright S. Size of populations and breeding structure in relation to evolution. Science. 1938;87:430–1.

28. Zerjal T, Xue Y, Bertorelle G, Spencer Wells R, Bao W, et al. The genetic legacy of the Mongols. Am J Hum Genet. 2003;72:717–21.

29. Balaresque P, Poulet N, Cussat-Blanc S, Gerard P, Quinana-Murci L, Heyer E, et al. Y-chromosome descent clusters and male differential reproductive success: young lineage expansions dominate Asian pastoral nomadic populations. Eur J Hum Genet. 2015;23:1413–22.

30. Falconer DF, McKay TF. Introduction to Quantitative Genetics. 4th ed. Essex: Pearson Education Limited; 1996. p. 464.

31. Frankham R. Effective population size/adult population size ratios in wildlife: a review. Genet Res. 1995;66:95–107.

32. Palstra FP, Ruzzante DE. Genetic estimates of contemporary effective population size: what can they tell us about the importance of genetic stochasticity for wild population persistence? Mol Ecol. 2008;17:3428–47.

33. Palstra FP, Fraser DJ. Effective/census population on size ratio estimation: a compendium and appraisal. Ecol Evol. 2012;2:237–2365.

34. Waples RS, Luickart G, Faulkner JR, Tallmon DA. Simple life-history traits explain key effective population size ratios across diverse taxa. Proc Biol Sci. 2013;280:20131339.

35. Wood AR, Esko T. Defining the role of common variation in the genomic and biological architecture of adult human

height. Nat Genet. 2014:10–1038.

36. Mueller E, Mazur A. Evidence of unconstrained directional selection for male tallness. Behav Ecol Sociobiol. 2001;50:302–11.

37. Zoledziewska M, Sidore C, Chiang CWK, Cucca F. Reducing variants and selection for short stature in Sardinia. Nat Genet. 2016;47:1352–6.

38. Ohta T. Slightly deleterious mutant substitutions in evolution. Nature. 1973;246:96–8.

39. Kimura M. The Neutral Theory of Molecular Evolution. Cambridge: Cambridge University Press; 1983. p. 384.

40. Frankham R, Jones LP, Barker JS. The effects of population size and selection intensity in selection for a quantitative character in Drosophila: I. Short-term response to selection. Genet Res. 1968;12:237–48.

41. Weber K. Population size and long-term selection. Plant Breed Rev. 2004;24:249–68.

42. The IUCN Red List of Threatened Species [Internet]. cited 2018. June 15. Available from: https://www.iucn.org/theme/species/our-work/iucn-red-list-threatened-species

43. Smith FA, Smith RE, Lyons SK, Payne JL. Body size downgrading of mammals over the late Quaternary. Science. 2018;360:310–3.

44. Hallman CA, Sorg M, Jongejans E, Siepel H, Hofland N. More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PLoS One. 2017;12:0185809–10.

45. Inger R, Gregory R, Duffy JP, Stott I, Vorisek P, Gaston KJ. Common European birds are declining rapidly while less abundant species' numbers are rising. Ecol Lett. 2015;18:28–26.

46. Bradford C, Garcia A. Climate-driven declines in arthropod abundance restructure a rainforest food web. Proc Natl Acad Sci USA. 2018: https://doi.org/10.1073/pnas.1722477115.

47. McRae L, Deinet S, Freeman R. The diversity-weighted living planet index: controlling for taxonomic bias in a global biodiversity indicator. PLoS One. 2017;12:e0169156.

48. Frankham R. Conservation genetics. Annu Rev Genet. 1995;29:305–27.

49. Frankham R, Ballou JD, Ralls K, Eldridge MD, Dudash MR, Fenster CB, et al. Genetic Management of Fragmented Animal and Plant Populations. Oxford. Oxford: Oxford University Press; 2017. p. 401.

50. Frankham R, Ballou JD, Eldridge MD, Lacy RC, Ralls K, Dudash MR, et al. Predicting the probability of outbreeding depression. Conserv Biol. 2011;25:465–75.

51. Ralls K, Ballou JD, Dudash MR, Eldridge MD, Fenster CB, Lacy RC, et al. Call for a paradigm shift in the genetic management of fragmented populations. Conserv Lett. 2018;11:1–6.

52. Fenster CB, Martén-Rodriguez S. Pollination specialization and the evolution of reproductive assurance mechanisms through autonomous selfing. Int J Plant Sci. 2007;168:215–28.

53. Ralls K, Harvey PH, Lyles AM. Inbreeding in natural populations of birds and mammals IN Smith JNM, Keller LF, Marr AB, Arcese P, editors. Conservation Biology: The Science of Scarcity and Diversity. Sunderland, MA:

Sinauer; 1986.

54. Pusey A, Wolf M. Inbreeding avoidance in animals. Trends Ecol Evol. 1996;11:201–6.

55. Hedrick PW. Genetics of Populations. 4th ed. Jones and Bartlett Publishers; 2011.

56. Alvarez G, Ceballos F, Quinteiro C. The Role of Inbreeding in the Extinction of a European Royal Dynasty PLoS One. 2009;4:10–1371.

57. East EM. Inbreeding in corn. Reports of the Connecticut Agricultural Experiments Station for. 1907;1907:419–28.

58. Shull GH. The composition of a field of maize. Reports of the American Breeders Association. 1908;296–301.

59. Dudash MR, Carr DE. Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems. Nature. 1998;393:683–4.

60. Johnson WE, Onorato DP, Roelke ME, Land ED, Cunningham M, Belden RC, et al. Genetic restoration of the Florida panther. Science. 2010;329:1641–5.

61. Barrett SC. The evolution of plant sexual diversity. Nat Rev Genet. 2002;3:274–84.

62. Wright S. The distribution of self-sterility alleles in populations. Genetics. 1939;24:538–52.

63. Lawrence MJ. The genetics of self-incompatibility in *Papaver rhoeas*. Proc R Soc Lond B Biol Sci. 1975;188:275–85.

64. Demauro MM. Relationship of breeding system to rarity in the Lakeside daisy (*Hymenoxys acaulis* var. *glabra*). Conserv Biol. 1993;7:542–50.

65. Florida Ziziphus Recovery Team. 2012. Florida Ziziphus Recovery Team Report. [Internet]. Cited 2018. June 15. Available from: http://www.archbold-station.org/html/research/plant/flziz.html

66. Dudash MR, Fenster CB. Inbreeding and outbreeding depression in fragmented populations IN Young A, Clarke G, editors. Genetics, Demography and Viability of Fragmented Populations. Cambridge: Cambridge University Press; 2000.

67. Fenster CB, Galloway 2000 LF. Inbreeding and outbreeding depression in natural populations of *Chamaecrista fasciculata* (Fabaceae): consequences for conservation biology. Conservation Biology. 14;1406–12.

68. Erickson DE, Fenster CB. Intraspecific hybridization and the recovery of fitness in the native legume *Chamaecrista fasciculata*. Evolution. 2006;60:225–33.

69. Frankham R. Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow. Mol Ecol. 2015;24:2610–8.

70. U.S. Fish and Wildlife Service. Mexican Wolf Recovery Plan, First Revision. Region 2, Albuquerque, New Mexico, USA. 2017.

71. Baldwin RF, Trombulak SC, Leonard PB, Noss RR, Hilty JA, Possingham HR, et al. The future of landscape conservation. Bioscience. 2018;68:60–3.