



Genetics does matter for population dynamics: Demographic implications of inbreeding depression in plants

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Abstract

Habitat fragmentation poses a serious threat for the viability of wild populations. The genetic effects of fragmentation on plant fitness components, such as inbreeding depression, are well known and the theoretical importance of genetic processes for population viability has extensively been studied. Conservation biologists need to develop approaches that enable us to integrate demography and genetics to ultimately assess the conservational status of plant species threatened by fragmentation of their habitats. In this paper, I address this issue by reviewing the genetic basis of inbreeding depression, the effects of inbreeding depression on plant performance, and the methods to quantify its effects on plant fitness components.

I. Introduction

The theoretical importance of genetic processes for population viability has extensively been studied during the last two decades [2, 7, 8, 40, 62, 63, 64, 70, 102, 116]. Conservation biologists are nowadays aware that both demographic (e.g. environmental and demographic stochasticity) and genetic (e.g. loss of genetic variability, random fixation of alleles by genetic drift, and inbreeding depression) processes act in concert to determine the viability of wild populations [116]. Even so, the amount of empirical evidence illustrating the effects of changing genetic processes on population viability is still scarce. The ongoing generalized fragmentation of plant populations due to human activities in many different habitats all over the world stresses the need to develop conservation plans that take into account the effects of genetic processes on viability of plant populations. Habitat fragmentation leads to decreasing population size and increasing isolation between extant populations. The most direct genetic consequence of fragmentation is that inbreeding (and/or biparental inbreeding) increases among individuals within populations resulting in a loss of heterozygosity and the expression of inbreeding depression, which is defined as the reduction in fitness of selfed progeny compared to outcrossed progeny [21, 23, 55]. For this reason, inbreeding depression is expected to be costly in demographic terms by increasing extinction probabilities.

Demographic models have proved to be very useful to conduct population viability analysis (PVA), which assesses mean time to extinction of populations mainly based on the effects of changing environment on demographic attributes [5, 41, 45, 73, 74, 97]. However, attempts to construct simulation models that jointly include demographic and genetic factors are not common given that genetic data on the species of interest are generally more difficult and expensive to obtain than demographic data [10]. Traditionally, genetic models emphasized only two types of genetics factors affecting extinction of small populations: inbreeding depression and the loss of genetic variability due to genetic drift [33, 34, 39, 50, 61]. We need to develop approaches that enable us to integrate demography and genetics to ultimately assess the conservational status of plant species threatened by fragmentation of their habitats [2, 82]. The goal of this paper is to provide evidence of the effects of inbreeding depression on fitness components of plants, and to stress the need to including that information into simulation models to assess the demographic implications of inbreeding depression. In the next sections, I briefly review the genetic basis of inbreeding depression. I then outline the methods to quantify the effects of inbreeding depression on plant fitness components. Finally, I discuss different ways to merge demography and genetics using both experimental and modeling approaches.

II. The genetic basis of inbreeding depression

Traditional genetic studies have clearly shown that inbreeding may affect several plant fitness components [21, 22, 23, 55]. In plants, the timing and magnitude of inbreeding depression on fitness components, and subsequently on the viability of populations, may strongly depend on mating system, such as selfing, mixed-mating and outcrossing systems [55, 65]. The general trend is that selfers express most of the inbreeding depression late in the life cycle, such as growth and reproduction of adult individuals, whereas outcrossers express substantial inbreeding depression at both early, such as seed development, and late life-cycle stages [55]. Despite general patterns, understanding the genetic basis of inbreeding depression is essential if we want to include genetic processes into demographic models to assert the demographic implications of inbreeding depression. There are three aspects that mainly determine the response of plants to inbreeding and the final effect of inbreeding depression on fitness components: first, the type of allelic interaction that can be of the dominant or overdominant type, second, the extent of epistasis that determine the interaction between loci, and finally, the number of loci and the distribution of their effects involved in the expression of inbreeding depression [16].

There are two main hypotheses that account for inbreeding depression [16, 21, 23]. Under the dominance hypothesis inbreeding depression results from the increase of recessive or partial recessive deleterious alleles that become expressed under homozygosity. Alternatively, under the overdominance hypothesis the decrease in fitness due to inbreeding depression is caused by the loss of heterozygotes that have higher fitness than homozygotes in the population. Most of the empirical work to determine the response of plants to inbreeding depression seems to indicate that the dominance hypothesis accounts for inbreeding depression in plants [4, 16, 21, 23, 99]. Nevertheless, some of these studies also suggest that in some cases the overdominance hypothesis cannot be ruled out completely [3, 29, 58]. Overdominance, however, can be difficult to detect because of a particular interaction between alleles of closely linked loci on opposite homologous chromosomes in a way that the dominant allele on one homologue complements the deleterious allele on the other [16].

Under the dominance hypothesis, purging of recessive deleterious alleles from the population is expected. In the case of an outcrossing plant population where selfing rates quickly increase due to fragmentation of the habitat, it would be expected that mean fitness of the population decreases at first, but after some generations of selfing, deleterious recessive alleles may have been purged from the population and mean fitness can equal or even exceed that of the initial outcrossing population [3, 14, 65]. Although the demographic implications of purging of recessive deleterious alleles seem to be many (e.g.

surviving individuals can increase performance under higher levels of inbreeding), empirical data suggest that most of the recessive deleterious alleles are hard to purge from the population [3, 23, 65, 88]. Basically, there are two main factors that prevent an effective purging. First, most of the loci responsible of inbreeding depression have a mild effect and loci of large effect (i.e. lethals and sublethals) on viability and fertility have only a small contribution to overall inbreeding depression [15, 29, 58, 113]. Hence, those individuals homozygous for loci of large effect are easily eliminated from the population whereas the rest survive maintaining substantial inbreeding depression even in high inbred populations [24, 25]. The other factor is that an association between loci determining mating system and inbreeding depression is required, as well as the coevolution of the two traits, but this point remains controversial.

A common result reported by several studies focusing on inbreeding depression in plants is the important between-individual variation in inbreeding depression [29, 55, 58, 79, 83, 87, 88, 94, 95]. Individuals within a population respond differently to pollination type in a way that some of them exhibit higher fitness under outcrossing whereas others perform much better under selfing. Different factors may account for individual-level variation in inbreeding depression: differences among individuals within the population in the number or recessive alleles that they carry as a result of their different history of inbreeding or in the accumulation of mutations [58, 100], the influence of maternal effects in determining the final response of individuals to inbreeding [14, 98], the interaction of different mechanisms and genes operating at different stages of the life cycle [17] or the associations that develop between loci determining mating system and loci determining different fitness traits [29, 79, 88]. The demographic implications of such a variation can be important since some individuals within the population might increase their performance under high levels of inbreeding reducing therefore the negative effects of inbreeding depression on population viability [88]. It remains to be seen, however, to what extent a small proportion of individuals performing better after repeated selfing can rescue the population from the general decreasing pattern in a high inbreeding scenario.

Understanding the relationship between the expression of inbreeding depression and increasing homozygosity is crucial if we want to quantify the demographic implications of inbreeding depression. Empirical studies to determine how fitness components change with increasing homozygosity of the genome have been performed to investigate the epistatic relationships among loci involved in inbreeding depression [20, 58, 59, 114], and maternal line improvement, which represents the mechanism for a selfing variant to invade a population [51, 52, 109]. At present, detecting epistasis has proved difficult to do [36] and in general there are three possible theoretical scenarios:

first, no epistasis where fitness decreases log-linearly with inbreeding load, second, reinforcing epistasis where the deleterious effect of homozygosity at a given locus becomes greater with increasing homozygosity, and third diminishing epistasis where the opposite occurs [16]. The viability of plant populations under high inbreeding may largely depend on the epistatic relationship among loci. Unfortunately, we still know little about epistasis among loci since detecting epistasis requires complex and time-consuming multigenerational inbreeding experiments [3, 29, 58, 72, 83, 111, 114].

III. The effects of inbreeding depression on plant performance

Inbreeding depression may greatly vary throughout the life cycle of plants and estimates on the response of plants to inbreeding depression at each life-cycle stage are needed to fully understand the demographic implications of inbreeding depression. Given the variety of life histories (e.g. annuals, short- and long-lived pluricarpic and monocarpic perennials) and growth forms (e.g. herbs, shrubs and trees) exhibited by plants [27], it results clear that obtaining estimates of inbreeding depression throughout all type of life cycles can be difficult or even impossible given the time required for some species to reach the reproductive status. However, recruitment is the limiting life-cycle phase of many long-lived perennials [18, 41, 42, 89, 101]. Hence, inbreeding depression estimates on early life-cycle stages (from seed set to seed germination and seedling survival) can suffice to assess the weight of inbreeding depression for population dynamics in these group of plants.

Analyzing the response of individual plants to inbreeding depression requires the experimental hand pollination of maternal plants in order to obtain selfed and outcrossed progeny. It must be noted that this applies for plants species that potentially can inbreed (e.g. hermaphrodites) and not for plants where inbreeding is no longer possible (e.g. dioecious plants). Hand-pollination experiments are interesting for two reasons. First, because the mating system of the species of study is discerned, and second, because it allows the growth of selfed and outcrossed progeny whose comparison provides the inbreeding depression coefficient for each life-cycle stage [1]. Mating system determines the mode of transmission of genes from one generation to the next. As a result, mating system is an important factor that affects levels of genetic variation and its distribution within and among populations [2, 11]. Moreover, knowing the mating system of the species is also important to have a complete overview on the non-demographic factors that may also influence the dynamics of plant populations. For example, autogamous plants can be more independent from pollinators than allogamous plants to avoid reproductive failure. Factors affecting pollinators, and that not

necessarily affect the plant of study, may have a pronounced effect on seed production rates which in turn would have repercussions on the population dynamics as a whole. In the case of fragmented landscapes, it has been demonstrated that fragmented sites have lower fruit production than non-fragmented sites as a result of the negative effects of fragmentation on pollinators [56, 78, 91, 96].

Hand-pollination experiments include two basic treatments: selfing (pollination of flowers with pollen of the same plant) and outcrossing (pollination of flowers with pollen of other plants). The addition of other treatments, such as one for the ability of self-fertilization, is always interesting to obtain more insights into the mating system of the species of study. Furthermore, the outcrossing treatment can in turn include two treatments, one with crosses between plants within the same population, and another between plants from other populations. This is especially interesting when populations of study are severely isolated due to fragmentation. Given that fragmentation increases the probability of inbreeding among plants within populations, gene flow (through seed and pollen) between populations can diminish the effects of fragmentation by increasing heterozygosity [30]. In fact, dispersal and connectivity between populations are important issues to understand the viability of fragmented populations [32, 33, 84]. Although hybridization between populations normally increases fitness, i.e. hybrid vigor, it may also decrease fitness, i.e. outbreeding depression [35]. If plants are highly adapted to their local environment, outcrossing between locally adapted plants can produce the disruption of genetic complexes, such as the case of strong epistatic interactions among loci that enhance fitness [31] or a combination of genetically based ecological differences [75]. It must be emphasized that local adaptation in plants is a common process and there are several examples suggesting the role of selfing in the process of population genetic differentiation [57, 71, 87, 90, 106, 108] that may lead to local adaptation.

Resulting selfed and outcrossed progeny have to be raised together in the same environment to compare the performance of plants from different pollination treatments from which inbreeding depression can be estimated. The magnitude of genetic factors, however, may strongly be influenced by the environment in which selfed and outcrossed progeny are compared [28, 108], and the effects inbreeding depression on plant traits increase under stressful competitive environments. For this reason, more detailed information can be obtained if the performance of selfed and outcrossed progeny is compared across different environments differing in the extent of harshness. The replication of the inbreeding depression experiment in different environments (e.g. greenhouse, common garden, and field conditions) also implies a considerable increase of the number of plants included in the experiment. In general, studies on inbreeding depression are conducted in greenhouse

conditions to control the environment and to avoid the loss of maternal lines. Limitations to replicate the experiment in other environments can be due to a limited number of offspring obtained per maternal plant and/or the difficulty to control undesirable sources of variation in the field.

Complementary, the use of molecular markers, such as microsatellites or enzyme systems, has proved to be very effective to determine mean heterozygosity of plant populations. Although, molecular markers cannot tell us what life-cycle traits are significantly affected by inbreeding depression, they can be used to investigate the relationship between population size and mean plant performance at the population level. Several studies have reported the relationship between genetic variation and population size [37, 68, 93, 107, 110, 115]. In general, small populations tend to have reduced heterozygosity and increased inbreeding coefficients, as predicted by theory. Due to the effects of inbreeding depression, reduced genetic variation and/or accumulation of deleterious mutations, populations with higher inbreeding coefficients also tend to exhibit lower fitness components [81, 85, 86, 110]. Hence, large-scale surveys and genetic analyses to determine the relationship between population size and mean inbreeding coefficient and between plant performance and genetic variation also contribute to assess the weight of genetic processes for population dynamics.

IV. Merging demography and genetics

Different attempts have been made to merge demographic and genetic data to investigate how genetic processes affect the dynamics of plant populations. From a theoretical point of view, different studies have analyzed the fate of populations affected by inbreeding depression using simulation models. Some models showed that inbreeding depression may induce extinction due to the positive interaction between inbreeding depression and reduction in population size [103, 104]. Extinction probabilities were even higher when stochastic variation in demographic attributes was included into the model [104]. These results provided support for the well-accepted theory that under particular conditions, populations can quickly enter into the so-called “extinction vortex” [43]. Other models pursued to identify the minimum viable population size that permits a given population to cope with the effects of inbreeding depression [46]. These models represent a very useful tool to understand the functioning of complex systems. However, although these models included important genetic (e.g. genomic mutation rate of lethals) and demographic (e.g. equilibrium population size, carrying capacity) parameters, they still remain too theoretical to be applied to real cases.

Other approaches tried to merge demography and genetics using experimental means. For example, experimental replicated populations of the annual plant *Clarkia pulchella* (Onagraceae) were established and monitored

during three generations [80]. Sets of populations differed in the relatedness of the founders, that is, in the genetic population size of the populations of origin. Results showed that mean fitness of plants in the high relatedness scenario was significantly lower than in the low relatedness scenario. These results also indicated that decreased genetic population sizes, which led to inbreeding and loss of alleles due to drift, significantly increased the probability of population extinction. Other studies on other plant species, such as *Arnica montana* (Asteraceae) [69] and *Succisa pratensis* (Dipsacaceae) [110], opted for the introduction of selfed and outcrossed seeds and seedlings, resulting from experimental crosses conducted in a greenhouse, into the populations of origin. These experiments evidenced that some plant traits exhibited significant inbreeding depression whereas others exhibited heterosis (i.e. the advantage of heterozygotes over homozygotes resulting from outcrossing). The take-home message of these studies was that restoration of inbred populations can be achieved by introducing outbred individuals into the populations, since population fitness, mate availability and attractiveness of pollinators to the populations increase. However, other studies found a great spatiotemporal variation in the amount of heterosis and hybrid breakdown suggesting that there may be great uncertainty in the long-term consequences of genetic manipulations of wild populations [36].

Although the examples above mentioned clearly suggest that genetic does matter for population dynamics, an approach fully based on field demographic data and inbreeding depression data for a particular species of study is still lacking. This has been identified as one of the common pitfalls of demographic genetic models [2, 82]. Hence, integrative approaches to obtain demographic (i.e. vital rates, such as survival, growth and reproduction) and genetic (i.e. effects of inbreeding depression on vital rates) data for a given plant species of interest should be developed. With both demographic and genetic data sets, a demographic model can be parameterized to analyze the demographic implications of inbreeding depression. Although many plant species may present serious limitations (e.g. particular complicated mating systems) to obtain accurate estimates of inbreeding depression, many others can be suitable to jointly conduct inbreeding depression experiments and demographic surveys, which can prove very useful to provide fundamental advances in this field.

Matrix population models represent the most accepted and widely used tools to analyze the demographic behavior of plant populations [19, 66, 67]. The advantage of using matrix models is that individual-based data can be used to parameterize the model to ultimately obtain important population-level parameters, such as population growth rate. This is important because field data in plant demography normally consist of life tables for a sub-sample of individuals containing the main demographic attributes of each individual

sampled. Life tables can be arranged in a matrix form and demographic parameters of the population can be derived by using well-known algebra algorithms [19]. Hence, survival and growth of individuals in different life-cycle stages (e.g. seedlings, juveniles and reproductive plants), and reproductive schedules of adults clearly corresponds to different matrix entries. Among other applications, stochastic modeling techniques can be applied using matrix models to simulate the effects of variation in environmental conditions on vital rates as well as to model density dependence or the carrying capacity of the system by including non-linear functions. The relative importance of each vital rate for the population, in terms of contributions to the population growth rate and to the spatiotemporal variance in population growth rate, can also be quantified by using elasticity [6, 19, 60] and variance decomposition analyses [9, 19, 53].

In an integrative approach, changes in population size over time represent the link between demography and genetics. Given the evident relationship between population size and probability of inbreeding, the extent of inbreeding depression on demographic traits can be related to population size. It must be noted that those demographic traits that are significantly affected by inbreeding depression can easily be identified from a crossing experiment for a given plant species. Hence, we know the demographic traits (or components of demographic traits) arranged in demographic matrices that will be affected by inbreeding depression. Specific functions can be parameterized for all matrix elements containing demographic traits affected by inbreeding depression, whose intensity depends in turn on variation in population size. Population- and individual-based approaches can be developed though they require different type of data. In particular, individual-based approaches are especially interesting to model different theories accounting for the genetic basis of inbreeding depression and to model the effect of individual-level variation in inbreeding depression.

It is clear that the more and better demographic and genetic data are available, the more accurate the outcome of the model will be. In fact, the lack of detailed data is one of the caveats of an integrative approach. It is well known that the response of plants to inbreeding depression may greatly depend on the interaction with the environment [28, 49, 108]. Hence, specific functions for vital rates relating different levels of inbreeding with variation in environmental conditions (e.g. water and nutrient availability, density dependence, herbivore effects) are required if we want to get more valuable insights into the interaction of demographic and genetic processes in the extinction of populations. Specific experiments, such as multigenerational inbreeding studies conducted in different environments, need to be designed to parameterize the existing models.

The final contribution of an integrative model including demographic and genetic data would be to gain more insights into the metapopulation dynamics of plants, that is, the consequences of migration, colonization and extinction events in spatially structured habitats [47, 77]. In a metapopulation context, the demographic implications of genetics are even more relevant and mating system and gene flow between populations become key processes. Populations of selfing plants can be founded by single individuals, so that the genetic composition of the founders could contribute to the reduced genetic variability within populations, phenomenon that would be comparable to the effects of severe bottlenecks [26]. In contrast, populations of outcrossing plants can also be founded by single individuals, but the success of the new population would depend more on gene flow between surrounding populations or on repeated successful colonization events over time. Demographic models including the effects of inbreeding depression on fitness components could also incorporate a spatial component if landscape data are obtained. Landscape data should include: the identification of suitable unoccupied patches within the landscape [48, 76, 92] the assessment of seed dispersal and pollen movement between populations [12, 105] and the effects of outcrossing with individuals from populations placed at different distances [13, 44, 112].

V. Conclusions

Given the important role that genetic processes (e.g. genetic deterioration and inbreeding depression) may play for the viability of plant populations in fragmented habitats, conservation plans can no longer omit the impact of genetics if conservation biologists want to prevent the extinction of wild populations [38, 40, 62]. The number of studies focusing on the demographic behavior of plants clearly outnumbers that focusing on the effects of inbreeding depression on fitness components. Thus, efforts must be directed towards increasing the information available on the mating system and the extent of inbreeding depression of plants occurring in fragmented landscapes. This applies not only to endemic plants but also to common plant species that have become rare because of human-induced habitat fragmentation, the so-called “new rares” [cf. 54, 82]. Different experimental and modeling approaches can be used to merge demography and genetics, not only to better understand the demographic effects of genetic processes and their ecological and evolutionary consequences, but to design effective conservation plans that assess the viability of plant populations.

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