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Do estimates of contemporary effective population size tell us what we want to know?

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Abstract

Estimation of effective population size (N_e) from genetic marker data is a major focus for biodiversity conservation because it is essential to know at what rates inbreeding is increasing and additive genetic variation is lost. But are these the rates assessed when applying commonly used N_e estimation techniques? Here we use recently developed analytical tools and demonstrate that in the case of substructured populations the answer is no. This is because the following: Genetic change can be quantified in several ways reflecting different types of N_e such as inbreeding (N_{eI}), variance (N_{eV}), additive genetic variance (N_{eAV}), linkage disequilibrium equilibrium (N_{eLD}), eigenvalue (N_{eE}) and coalescence (N_{eCo}) effective size. They are all the same for an isolated population of constant size, but the realized values of these effective sizes can differ dramatically in populations under migration. Commonly applied N_e-estimators target N_{eV} or N_{eLD} of individual subpopulations. While such estimates are safe proxies for the rates of inbreeding and loss of additive genetic variation under isolation, we show that they are poor indicators of these rates in populations affected by migration. In fact, both the local and global inbreeding (N_{eI}) and additive genetic variance (N_{eAV}) effective sizes are consistently underestimated in a subdivided population. This is serious because these are the effective sizes that are relevant to the widely accepted 50/500 rule for short and long term genetic conservation. The bias can be infinitely large and is due to inappropriate parameters being estimated when applying theory for isolated populations to subdivided ones.

KEYWORDS
50/500 rule, additive genetic variance, inbreeding, isolation, metapopulation effective size, N_e estimation migration, substructured populations

1 | INTRODUCTION

Maintaining high levels of genetic diversity and keeping inbreeding low are important aspects in the management of threatened populations. For this reason the concept of genetically effective population size (N_e) plays a central role in conservation biology; N_e relates to the rate at which genetic drift occurs, and in particular the rates of inbreeding and loss of genetic diversity are of concern in conservation. The past decade "has seen an explosion of interest in the use of genetic markers to estimate effective population size" (Waples, 2016). Little attention has been paid, however, to whether those estimates really quantify the relevant rates of genetic change when substructured populations are the focus of empirical studies.

Effective population size was originally defined for a single, isolated population of constant size (Wright, 1931), and N_e can be viewed as the size of an idealized population with nonoverlapping generations (a so-called Wright-Fisher population) with the same properties of genetic drift as the population at hand (Gilbert & Whitlock, 2015). There are many ways to describe and quantify genetic drift, however, and a series of different N_e relating to different aspects of the drift process have been proposed. Wright's (1931) initial work focused on quantifying the rate of inbreeding (i.e., increase in homozygosity of alleles that are identical by descent), and this quantity is denoted inbreeding effective size (N_{eI}). Subsequently, effective sizes that quantify other parameters have been defined. They include the variance effective size (N_{eV}) that relates to the amount of allele frequency change, the additive genetic variance effective size (N_{eAV}) that quantifies the rate at which additive genetic variation is lost, the coalescence effective size (N_{eCo}) that indicates the rate at which present alleles in the population can be traced back to common ancestors, and the eigenvalue effective size (N_{eE}) that corresponds to the effective size when equilibrium has been attained and the rate of inbreeding is constant (Table 1; Hössjer, Laikre, & Ryman, 2016; Jorde & Ryman, 1995,2007; Lynch & Walsh, 1998; Sjödin, Kaj, Krone, Lascoux, & Nordborg, 2005; Waples, 1989; Whitlock & Barton, 1997). In the simplest case of an isolated population of constant size these effective sizes are, by definition, all the same, and the processes they quantify can, if viewed back in the population genealogy (genetic history), be regarded as the distribution of times to common ancestry among current gene copies in the population (the coalescent; appendix 10 in Allendorf, Luikart, & Aitken, 2013).

Most natural populations are not completely isolated, however, but connected to others by more or less frequent migration. In contrast to the situation with isolated populations various types of N_e can be very different for a population under migration (Chesser, Rhodes, Sugg, & Schnabel, 1993; Wang, 1997a,1997b). Considerable work has been devoted to modelling effective sizes of subdivided populations (e.g., Maruyama & Kimura, 1980; Nunney, 1999; Tufto & Hindar, 2003; Wang & Caballero, 1999; Waples, 2010; Whitlock & Barton, 1997; Wright, 1938). Most of these efforts, however, have focused on a single effective size (N_{eI} or N_{eV}) using simplifying assumptions such as drift-migration equilibrium, haploid populations, or ideal demographic conditions where census and effective sizes under isolation are identical ($N_c = N_e$). Means for modelling several types of N_e under both equilibrium and nonequilibrium conditions and for complex metapopulations deviating from nontraditional patterns of migration have previously not been possible.

We have recently developed a general analytical framework for exploring the dynamics of many effective population sizes in more complex metapopulations (Hössjer et al., 2016; Hössjer, Olsson, Laikre, & Ryman, 2014,2015). Our approach allows modelling systems at equilibrium as well as before equilibrium has been reached, with any number of subpopulations of arbitrary census and effective size under isolation. Migration patterns are also optional, as are initial degrees of inbreeding and relatedness within and among populations. As an example, we applied this analytical tool to model the case of the wolf metapopulation on the Fennoscandian peninsula and showed that the observed unidirectional gene flow from Finland to Sweden greatly reduces the overall metapopulation inbreeding effective size. Further, gene flow from a large Russian wolf population into the Fennoscandian metapopulation has limited effect on inbreeding rates unless gene flow within Fennoscandia increases substantially (Laikre, Olsson, Jansson, Hössjer, & Ryman, 2016). These observations were previously unknown phenomena of direct relevance to management.

The "50/500 rule" of Franklin (1980) presents an example of a situation where it may be critical to know the particular type of N_e that is obtained when applying an estimator to genotypic data. This rule has become widely established in conservation biology, suggesting that for a single isolated population $N_e \geq 50$ is needed for short-term conservation and $N_e \geq 500$ for long-term conservation (Allendorf et al., 2013; Franklin, 1980). As detailed by Franklin (1980) the short-term rule of $N_e \geq 50$ refers to an effective size quantifying the rate of inbreeding (inbreeding effective size, N_{eI}). The logic of the 50-rule is that too rapid inbreeding can result in excessive homozygosity for deleterious recessive alleles resulting in inbreeding depression and reduced fitness (Chapter 10 of Lynch & Walsh, 1998). An $N_{eI} \geq 50$ implies that inbreeding increases by no more than 1% per generation, which is considered acceptable with respect to fitness over short time periods (Franklin, 1980). The long-term "$N_e \geq 500$ rule" refers to an effective size relating to loss of additive genetic variation, here referred to as N_{eAV} (Hössjer et al., 2016; Table 1; below), and the concern here is the maintenance of sufficient levels of genetic variation for quantitative traits associated with fitness that will allow adaptation to new selective regimes (i.e., retention of evolutionary potential). Indeed, it follows from Fisher's Fundamental Theorem of Natural Selection (Price, 1972) that it is the amount of additive genetic variance that will determine the rate of fitness change. With $N_{eAV} \geq 500$ the loss of such variation through drift is considered to be compensated for by new mutations (Allendorf & Ryman, 2002; Franklin, 1980).

Obtaining empirical estimates of effective size is crucial in the management of natural animal and plant populations to find out, e.g., if a particular population reaches any of the targets of the 50/500 rule. Rapidly growing efforts have been devoted to developing and applying methods that are based on genetic markers for estimating contemporary N_e in natural populations; such estimates are used to provide practical conservation management advice (e.g., Harris et al., 2017; Kajtoch, Mazur, Kubisz, Mazur, & Babik, 2014; Rieman & Allendorf, 2001; Sarno, Jennings, & Franklin, 2015; Wennerström, Jansson, & Laikre, 2017), and several papers discuss and compare the performance of various approaches (e.g., Gilbert & Whitlock, 2015; Luikart, Ryman, Tallmon, Schwartz, & Allendorf, 2010; Palstra & Ruzzante, 2008; Wang, 2005; Wang, 2016; Waples, 2016).

However, there are several problems associated with estimation of N_e in populations that are not isolated. Current methods for assessing N_e of subdivided populations are typically based on the assumption of isolation. Migration is dealt with as a complicating factor that creates a bias for the effective size, a bias that should

TABLE 1 Definition/description of symbols used in this paper

Symbol	Definition/comments
s	Number of subpopulations
t	Time measured in generations
N_c	Census population size
N_e	Effective population size (in general)
x	An arbitrary subpopulation that is part of a metapopulation
f	Coefficient of inbreeding
f_x	Average inbreeding in subpopulation x
f_{Meta}	Average inbreeding coefficient of the total metapopulation (here weighted according to subpopulation effective size). Corresponds to f_I in Hössjer et al., (2015)
m	Migration rate, in the context of an island model, expressed as the proportion of individuals in each generation that are immigrants from the metapopulation as a whole (including the target population). Migration is stochastic and m reflects the binomial average
m'	Migration rate, expressed as the proportion of individuals in each generation that are immigrants from *outside* the target population. Migration is stochastic and m' reflects the binomial average. In an island model, where immigrants can be conceptualized as drawn from an infinitely large pool of individuals to which all the s subpopulations have contributed equally, m and m' are related as $m' = m(s–1)/s$. Many texts on the island model are not explicit when defining migration, i.e., it is not always clear whether or not the immigrants include a proportion of individuals from the target population
N_{eI}	Inbreeding effective size (in general). N_{eI} reflects the rate at which inbreeding increases; inbreeding is the occurrence of homozygosity of alleles that are identical by descent, i.e., alleles that can be traced back to the exact same allele copy in an ancestor (also known as the coalescent). N_{eI} is not defined for situations where inbreeding decreases, and in a case where inbreeding stays constant we have $N_{eI} = \infty$
N_{eV}	Variance effective size (in general). N_{eV} reflects the rate of allele frequency change. The quantity of interest is the change of the standardized drift variance
N_{eLD}	Linkage disequilibrium effective size (in general); it reflects the degree of linkage (gametic phase) disequilibrium. Mathematical treatment of N_{eLD} is complicated and not yet fully resolved. Approximate equations for N_{eLD} in a local population exist for the special case of an ideal ($N_{ex} = N_{cx}$) island model (Waples & England, 2011; this paper) but not for the global population
N_{eGD}	Gene diversity effective size (in general). This quantity reflects the rate at which gene diversity, i.e., expected heterozygosity, declines. We have previously (Hössjer et al., 2016) referred to this N_e as "haploid inbreeding effective size," but here we call it N_{eGD} to avoid confusion in the present context
N_{eAV}	Additive variance effective size (in general); it reflects the rate at which additive genetic variation is lost due to genetic drift. N_{eAV} is very close to N_{eGD}, and in this paper we have used N_{eGD} (which is easier to compute) as a proxy for N_{eAV}
N_{eCo}	Coalescence effective size (in general); it reflects the time for ancestral lineages to coalesce to a common ancestor. We have not focused on this effective size; see the Discussion for more details on this N_e including its relationship to other N_e dealt with here
N_{cx}	Census size of subpopulation x
N_{ex}	Effective size of subpopulation x in isolation, i.e. when all types of N_e are the same, i.e. $N_{ex} = N_{eI} = N_{eV} = N_{eLD} = N_{eAV}$ etc
N_{eIRx}	Inbreeding effective size of subpopulation x (under prevailing migration scheme)
N_{eVRx}	Variance effective size of subpopulation x (under prevailing migration scheme)
N_{eLDRx}	Linkage disequilibrium effective size of subpopulation x (under prevailing migration scheme)
N_{eAVRx}	Additive variance effective size of subpopulation x (under prevailing migration scheme)
N_{eE}	Eigenvalue effective size (of the metapopulation as a whole). The global population will eventually reach a state where inbreeding increases at a constant rate, which results in the inbreeding effective size of the metapopulation to stay constant at a value indicated by N_{eE}. In a metapopulation where each subpopulation exchanges migrants with the rest of the system (through one or more subpopulations) the rate of inbreeding will eventually be the same ($1/(2N_{eE})$) in all subpopulations as well as for the system as a whole
N_{eMeta}	Total effective size (in general) of the metapopulation as a whole (the global population)
N_{eIMeta}	Total (global) inbreeding effective size of the metapopulation as a whole. This quantity reflects the change of f_{Meta} from generation t to $t + 1$. N_{eIMeta} can be viewed as a weighted average of N_{eIRx} over all subpopulations, and it will eventually approach N_{eE}.
N_{eVMeta}	Total (global) variance effective size of the metapopulation as a whole. N_{eVMeta} eventually approaches a value very close to N_{eE}, but N_{eVRx} of a local population does not (cf. Hössjer et al., 2016)
$N_{eLDMeta}$	Total (global) linkage disequilibrium effective size of the metapopulation as a whole. Currently, analytical as well as simulation approaches to assess this parameter are missing. In the present paper we only deal with the local form N_{eLDRx}
$N_{eAVMeta}$	Total (global) additive variance effective size of the metapopulation as a whole

be removed as thoroughly as possible (Wang & Whitlock, 2003). Gilbert and Whitlock (2015), for example, evaluated computer programs estimating effective size, and when considering populations experiencing migration they ranked the programmes according to their ability to accurately estimate N_e as it would be if the population

were completely isolated. The estimates that performed well in this ranking can be properly interpreted if the targeted population is really isolated, thus referring to a situation where all types of N_e are identical and an estimate of one type can be used as a substitute for another. However, recognizing the rates of genetic change in a population if it had been isolated, when in reality it is not, is suboptimal in practical conservation. Rather, it is central to understand the effects on the rate of inbreeding from conservation efforts such as maintaining/creating migration corridors to facilitate genetic exchange between populations (Atickem et al., 2013; Bennett, 1990; Cannas, Lai, Leone, & Zoppi, 2018; Ramiadantsoa, Ovaskainen, Rybicki, & Hanski, 2015).

Further, the most widely used estimators of N_e from genotypic data target N_{eV} or N_{eLD} (Gilbert & Whitlock, 2015). For isolated populations of constant size such estimates can be directly translated into N_{eI} or N_{eAV} and thus provide the rates at which inbreeding increases or additive variance is lost – the rate of particular relevance to conservation. In contrast, this may not be the case for populations under migration. Overall, the issue of which effective sizes that are estimated empirically in substructured populations when applying different estimators has, as far as we are aware, not been addressed.

In this paper we focus on exploring how N_{eV} and N_{eLD} relate to N_{eI} and N_{eAV} in metapopulations. We address the following questions: (a) When do different types of effective size follow the same dynamics to the extent that they can be used as approximate substitutes for one another in substructured populations, and how much do their dynamics differ otherwise; and (b) What is the expected magnitude of bias when using estimates of N_e obtained under the assumption of isolation in situations where this conjecture is erroneous?

We find that frequently applied estimators of N_e do typically not reflect the rates of inbreeding or loss of additive genetic variation of separate subpopulations in the face of migration. We conclude that estimates of contemporary N_e from empirical data do not tell us what we need to know for efficient conservation management.

2 | MATERIALS AND METHODS

Focusing on the effects of migration and drift (ignoring mutation and selection) we use our newly developed theory (Hössjer et al., 2016; Hössjer, Olsson, Laikre, & Ryman, 2014,2015) to describe the simultaneous expected change of several effective sizes of subpopulations within a metapopulation as the system evolves towards migration-drift equilibrium, paying particular attention to those relevant to the 50/500 rule (N_{eI} and N_{eAV}; Franklin, 1980). We compare these parametric (true) values of N_{eI} and N_{eAV} with those expected to be obtained when estimating contemporary effective size from genetic marker data using the "temporal method" that assesses N_{eV} from temporal shifts of allele frequencies and the one that uses linkage disequilibrium (LD) for estimation (N_{eLD}; below).

2.1 | Conceptual background

The genotypic distribution of a subpopulation in a metapopulation is affected by both genetic drift and migration. Thus, all types of N_e such as inbreeding (N_{eI}) and variance (N_{eV}) effective size are no longer the same (as they are in an isolated population). For each form of effective size we have an N_e of the metapopulation as a whole (N_{eMeta}) in addition to the N_e of each of the separate local populations. Further, N_e will change as the system approaches migration-drift equilibrium, and the rate of approach may differ among subpopulations.

There is some confusion in the literature regarding the effective size of a subpopulation that is part of a metapopulation. Subpopulation effective size has either been reserved to describe the genetic dynamics under ideal conditions had the subpopulation been isolated (e.g., Gilbert & Whitlock, 2015) or has been used to also include the effects of migration, mutation, and selection (Durrett, 2008; Ewens, 1989,2004; Hössjer, Olsson, Laikre, & Ryman, 2014, 2015; Wang, 1997a,1997b). For example, should variance effective size (N_{eV}) of a local population reflect actual allele frequency shifts resulting from the combined forces of drift, migration, mutation, and/or selection, or should it just signify the effects of sampling (genetic drift) within the population, i.e., the N_{eV} as it would be under complete isolation?

In this paper we use the wider approach and consider the joint effects of drift and migration when defining effective size of a local population in a metapopulation. We follow the nomenclature of Laikre et al. (2016) and Olsson, Laikre, Hössjer, and Ryman (2017) and make a distinction between N_e of a local population "x" under isolation (N_{ex}, which is the same for all types of genetic drift) and the *realized* effective size of subpopulation *x* when the joint effects of drift and migration are taken into account (e.g., N_{eIRx} or N_{eVRx}; Table 1). We note here that realized effective size is the quantity being estimated when sampling from a local population under migration and applying an unbiased estimator. For example, the temporal method estimates N_{eVRx} of subpopulation *x* (below). We also note that the metapopulation as a whole is thought to be isolated without immigration from other sources, implying that "realized N_e" only refers to local subpopulations, whereas migration between subpopulations is always included when considering the total metapopulation effective size (N_{eMeta}).

We argue that using the wider definition of realized effective size is crucial for relevance to conservation. In many situations it is by no means obvious that an investigator dealing with a population experiencing migration is primarily interested in knowing what N_e would be in the hypothetical situation of isolation. In the context of short-term conservation it could be more appropriate to ask for an estimate of N_{eIRx} that reflects the contemporary rate of inbreeding (including the effects of migration) rather than its expected equivalent under isolation. Similarly, the type of effective size assessed by the estimator applied (say, N_{eVRx}) may be a poor substitute for the type relevant to the biological question at hand, e.g. N_{eIRx}, when dealing with a population under migration. We are not aware, however, of any attempts

to quantify the bias that may result from such approximations, a task implying assessment of the simultaneous change of multiple forms of effective sizes in a spatially structured population, and we perform such analyses here.

2.2 | Types of N_e considered

We consider the dynamics of effective sizes referring to inbreeding (N_{eI}), variance (N_{eV}), linkage disequilibrium (N_{eLD}), eigenvalue (N_{eE}), and additive genetic variance (N_{eAV}) and some of their characteristics are described briefly below (notations in Table 1).

For a diploid organism the coefficient of inbreeding (f) is the average inbreeding coefficient (over individuals) in the population considered. The *inbreeding effective size* in generation t is defined as $N_{eI} = 1/(2\Delta f)$, where $\Delta f = (f_t - f_{t-1})/(1 - f_{t-1})$ and f_t is the inbreeding coefficient in generation t. The inbreeding coefficient provides the probability of identical homozygosity in a randomly chosen locus in a random individual in the population at a specific time point. In a diploid population, Δf is also related, but not equivalent to, the probability per generation that two alleles in an individual coalesce within a few generations back from t (Hössjer et al., 2014, 2015; Whitlock & Barton, 1997). In an isolated population of constant size, Δf is constant and exclusively determined by drift. In contrast, in a local population receiving immigrants, Δf is determined by both drift and immigration and the corresponding N_e is the realized effective size (N_{eIRx}).

The inbreeding effective size of the total metapopulation (N_{eIMeta}) is defined as for a local population except that f now refers to the (weighted) average inbreeding of the metapopulation as a whole; it corresponds to the weighted harmonic average of the N_{eIRx} of the different subpopulations. N_{eIMeta} can be computed using various schemes for weighting the separate fs of the different subpopulations such as local effective or local census (N_c) size (Hössjer et al., 2014,2015). In a traditional island model all the local N_{eIRx} will coincide with N_{eIMeta}, because all the subpopulations are of equal size and have the same expected N_{eIRx}, but this simple relationship does not hold for more complicated migration models (e.g., the linear stepping stone model in Figure 4).

The *variance effective size* (N_{eV}) relates to the amount of allele frequency change due to local genetic drift and migration, and the quantity of interest is the change of the standardized drift variance (e.g., Jorde & Ryman, 1995,2007; Waples, 1989). This variance can be conceptualized through considering an infinite number of isolated replicate populations of the same size and the same initial frequency of a particular allele. In a later generation allele frequencies have drifted apart, and the variance of allele frequencies among the replicate populations is defined as the drift variance (standardized with respect to the starting allele frequency) of that particular generation (Jorde & Ryman, 1995). The variance effective size of the total metapopulation (N_{eVMeta}) reflects the change of the weighted mean allele frequency of the different subpopulations.

Hössjer et al. (2016) considered a quantitative trait where the genetic component of the phenotypic variation is determined by multiple loci with additive effect, and derived an expression for an *additive genetic variance effective size* (N_{eAV}). This effective size reflects the rate at which additive genetic variation decays over time as a function of population size, due to local genetic drift and migration. It thus corresponds to the $N_e \geq 500$ rule for long-term conservation. Hössjer et al. (2016) also showed that N_{eAV} is very close to an effective size describing the decay of gene diversity (N_{eGD}), originally referred to as "haploid inbreeding effective size" by Hössjer et al. (2016), Hössjer et al. (2014), Hössjer et al. (2015)), which is computationally easier to assess.

The *eigenvalue effective size* (N_{eE}; Ewens, 1982; Tufto & Hindar, 2003; Hössjer et al., 2014; Hössjer, 2015) corresponds to the effective size of the metapopulation as a whole when migration-drift equilibrium has been attained. There are actually two forms of N_{eE}, a haploid one relating to allele frequencies and a diploid one associated with genotypic frequencies. The difference between them is generally negligible, however (Hössjer et al., 2015), and here we make no distinction between them and only give values for the diploid form. In a metapopulation where each subpopulation both receives immigrants from, and sends emigrants to, the rest of the system (through one or more subpopulations) the rate of inbreeding will eventually be the same ($1/[2N_{eE}]$) in all subpopulations as well as for the system as a whole (Hössjer et al., 2014, equation 61; Hössjer et al., 2015, equation 49).

A general theory for the linkage disequilibrium (LD) effective size (N_{eLD}) for subdivided populations is still lacking, but Waples and England (2011) presented approximate expressions for some specific situations of an island model at migration-drift equilibrium. They focused on randomly recombining loci, which means that N_{eLD} quantifies effective size of the recent past, a few generations back in time. Waples and England (2011) derived formulas for the major components contributing to LD (drift and mixture) in a subpopulation. They only considered the two special cases of an island model with two or 10 subpopulations, however, and they did not present a direct equation for N_{eLDRx}. We expand their analysis and provide an explicit approximate expression for subpopulation N_{eLDRx} that does not assume migration-drift equilibrium and that applies to an arbitrary number of subpopulations (Supporting Information Appendix S1, equation 29). This N_e corresponds to the size of an ideal population, where the forces affecting LD (drift and recombination) between unlinked loci are in balance, which has the same amount of expected LD as that observed in the focal population. We consider only the dynamics of subpopulation LD effective size (N_{eLDRx}) since analytical expressions for metapopulation N_{eLD} are still missing.

2.3 | Estimating N_e from empirical data

A large number of approaches and computer programs are available for estimating effective size from genetic marker data (reviews by e.g., Gilbert & Whitlock, 2015; Luikart et al., 2010; Palstra & Ruzzante, 2008; Wang, 2005,2016). Until recently, most studies were based on the "temporal method" that compares allele frequencies in samples collected one or more generations apart

to assess variance effective size (N_{eV}; e.g., Jónás, Taus, Kosiol, Schlötterer, & Futschik, 2016; Jorde & Ryman, 1995,2007; Nei & Tajima, 1981; Wang & Whitlock, 2003; Waples, 1989). During the past decade, however, estimation procedures that only require a single sample, collected at one point in time, have become prevailing (Palstra & Fraser, 2012; Waples, 2016). Among these one-sample estimators the method that assesses N_e from linkage disequilibrium (N_{eLD}; e.g. Do et al., 2014; Hill, 1981; Waples, 2006; Waples & Do, 2010) was the recommended one in a recent review of methods for estimating effective size, and most investigators seem to prefer this approach when appraising N_e from a single sample (Gilbert & Whitlock, 2015).

2.4 | Analytical approach

We consider the island and linear stepping stone models of migration, a nonselfing diploid organism with discrete generations, and describe the simultaneous expected change of local and global effective sizes of N_{eI}, N_{eV}, and N_{eAV}, the metapopulation N_{eE}, and N_{eLD} of the local populations during the approach to migration-drift equilibrium. Mating occurs after migration, and migration is stochastic such that rates reflect the binomial average. Migration rates are expressed either as the actual number, or as the proportion (m'), of immigrants per generation. For an island model, where immigrants originate from the global population "as a whole", some texts include the target population in "as a whole" whereas others do not. In this paper we let m and m' signify the situations where the global population "as a whole" includes and excludes the target population, respectively. Thus, in an island model with s subpopulations we have $m = m' \times s/(s-1)$, while m' is the only relevant quantity under the stepping stone model (cf. Table 1).

All metapopulations considered include 10 subpopulations of constant size with $N_{ex} = N_{cx} = 50$.

Initial inbreeding and kinship is zero (0) within and between populations, and we disregard the forces of selection and mutation. The expected N_e trajectories were calculated using analytical developments of Hössjer et al. (2014, 2015, 2016); key expressions applied for N_{eI} include equation 25 in Hössjer et al. (2014), equation 48 in Hössjer et al. (2015), and equation 29 in Hössjer et al. (2016). Expressions for N_{eV}, N_{eE}, N_{eAV} are equations 26, 36, and 30–32 of Hössjer et al. (2016), respectively. The GESP computer program (Olsson et al., 2017) was employed for some of the calculations.

We used equation 29 in the Supporting Information Appendix S1 when calculating the expected value of N_{eLDRx} in a local population of an island migration model. There is no theory for the behaviour of N_{eLDRx} under the stepping stone, and we employed a simulation approach to assess "expected" values under this migration model. We used the EASYPOP simulation program (Balloux, 2001) to generate genotypic distributions under the linear stepping stone and the required number of generations. We considered a diploid organism with two sexes and an equal sex ratio, 500 biallelic loci, no mutations, and we used the "maximal variability" option for genetic variation in the starting generation. The output files from EASYPOP were analyzed using the LD method of Waples and Do (2008) as implemented in the software NeEstimator V2 (Do et al., 2014), screening out alleles segregating at a frequency less than 0.05 (Pcrit = 0.05); final estimates of N_{eLDRx} were taken as the harmonic mean from 100 replicate runs (subpopulations).

3 | RESULTS

We find that various forms of local and global effective size exhibit quite divergent behaviours in populations under migration, and the general relationship between the different forms of N_e is similar under the island and the linear stepping stone migration models.

3.1 | Island model

The change of local and global effective sizes during approach to migration-drift equilibrium for the island model with a migration rate of one individual per generation is shown in Figure 1. The identical size of local populations and the symmetrical migration scheme imply that all local realized N_e are identical for each particular type of effective size, and that some types of N_e behave in a similar way. All the 10 N_{eIRx} are the same, for example, and they coincide with N_{eIMeta} that represents a weighted harmonic average of the local N_{eIRx}. At equilibrium they all converge on the eigenvalue effective size, $N_{eE} = 605$, and they are very close to this value after about $t = 150$ generations. The realized additive genetic variance effective size of a local population (N_{eAVRx}) is also very similar, but not identical to, the N_{eIRx}.

The most important observation refers to the different behaviors of the local realized effective sizes N_{eIRx} and N_{eAVRx} on one hand, i.e., those relating to the 50/500 rule in conservation, and those of N_{eVRx} and N_{eLDRx} on the other, i.e. those that are typically targeted when estimating effective size from genetic marker data (Figure 1). Clearly, applying either of the temporal or LD methods, which estimate N_{eVRx} and N_{eLDRx}, respectively, will tell us very little about rates of inbreeding (N_{eIRx}) or potentials for maintaining genetic variation (N_{eAVRx}) in local populations that are part of a metapopulation system. The trajectories of N_{eVRx} and N_{eLDRx} change only marginally during the first few generations such that N_{eVRx} decreases slightly and N_{eLDRx} increases. Then they reach equilibrium and stay indefinitely just under/over their original values of $N_{ex} = 50$; i.e., at $t = 500$ we have $N_{eVRx} = 49.0$ and $N_{eLDRx} = 51.9$.

With respect to the global population, the dynamics of the variance and additive genetic variance effective sizes (N_{eVMeta} and $N_{eAVMeta}$) are very similar, but not identical. They both start out at $N_e = 500$ (the sum of the local N_{ex}) and converge, at marginally different rates, on $N_{eE} = 605$. Before equilibrium has been approached $N_{eAVMeta}$ is a poor indicator of the rate of decay of additive genetic variation in the local populations, which is quantified by N_{eAVRx}.

Increasing migration to ten individuals per generation ($m = 0.22$; $m' = 0.20$) reveals a pattern that is qualitatively very similar to that for $m = 0.022$ (Figure 2 vs. Figure 1). The major difference is that

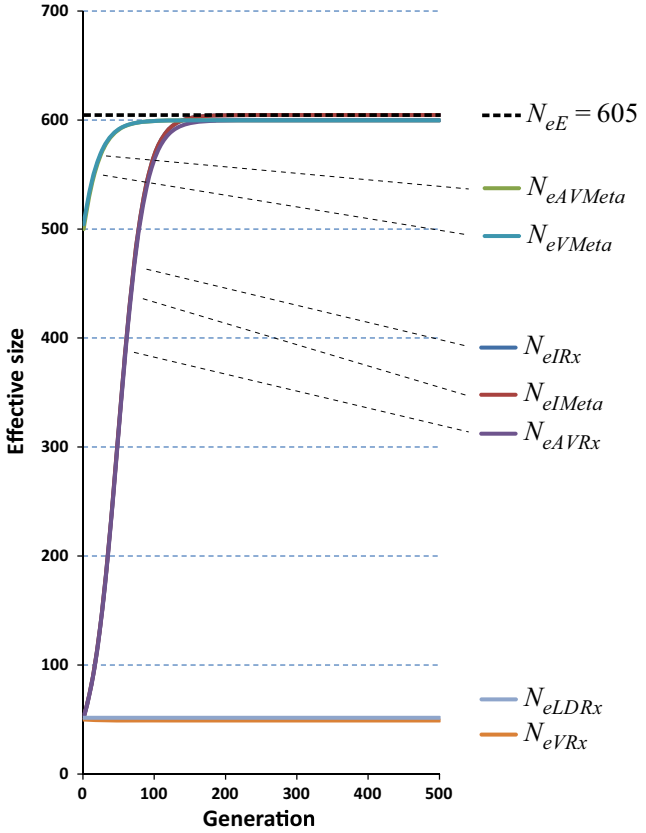

FIGURE 1 Global (*Meta*) and realized local (*Rx*) effective population sizes over 500 generations in a metapopulation following an island model pattern of migration. There are ten (10) ideal subpopulations of constant effective size $N_{ex} = N_{cx} = 50$, and in every generation each subpopulation receives on average one (1) immigrant drawn at random from an infinitely large migrant pool to which the other subpopulations have contributed equally ($m' = 0.02$; $m = 0.022$). N_{eI} relates to the rate of inbreeding, N_{eAV} to the rate at which additive genetic variation is lost, N_{eV} to of the amount of allele frequency change, and N_{eLD} reflects the degree of linkage disequilibrium resulting from a balance between genetic drift and recombination. The eigenvalue effective size is $N_{eE} = 605$, reflecting the equilibrium state when inbreeding increases at the same constant rate globally as well as locally resulting in $N_{eE} = N_{eIMeta} = N_{eIRx}$. Initial inbreeding and kinship is zero (0) within and between all subpopulations. Note that expected genetic change is the same for all subpopulations under an island model

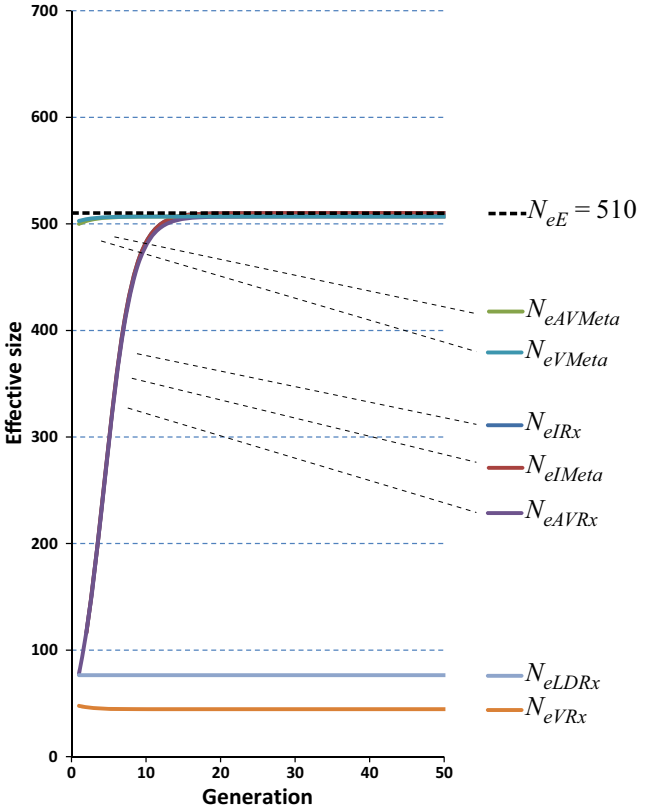

FIGURE 2 As in Figure 1 except that immigration rate is ten (10) individuals per generation ($m' = 0.20$; $m = 0.22$) and the process is only followed over 50 generations. The eigenvalue effective size is $N_{eE} = 510$

the higher migration rate results in a faster approach to equilibrium (note the different *x*-axis scales of Figure 2 vs. Figure 1). Further, the trajectories for N_{eVRx} and N_{eLDRx} level out at values that are more distant from the starting point ($N_{ex} = 50$) than at the lower migration rate. $N_{eVRx} = 44.5$ in generation $t = 50$ (compared to $N_{eVRx} = 49.0$ in Figure 1). For N_{eLDRx}, the expected local equilibrium value has increased from $N_{eLDRx} = 51.9$ (at $m = 0.022$; Figure 1) to $N_{eLDRx} = 77.2$ (at $m = 0.22$; Figure 2). In contrast to the simulations with $m = 0.022$ (Figure 1), simulated values with $m = 0.22$ are a bit high, in the range 81–87, rather than close to the expected value of 77.2 (Supporting Information Appendix S1). At large, however, the lack of coupling persists between the quantities relating to the 50/500 rule on one hand, and those estimated in most empirical studies on the other.

3.2 | Island model equilibrium conditions

Figure 3 depicts the equilibrium values at different migration rates (m) for the local forms of N_{eIRx}, N_{eVRx}, and N_{eLDRx} in an island model metapopulation with the same basic demography as previously, ($s = 10$, $N_{ex} = N_{cx} = 50$). Thus, comparing the curves in Figure 3 with those in Figure 2, for example, the equilibrium values for $m = 0.22$ are $N_{eIRx} = 510$, $N_{eVRx} = 44.5$, and $N_{eLDRx} = 77.2$. When m is small, say, $m < 0.10$, the expected local equilibrium values of N_{eVR} and N_{eLDR} are close to those in isolation when all local N_e are the same ($N_{ex} = 50$). An unbiased estimator targeting N_{eVRx} or N_{eLDRx}, such as methods based on the temporal or the LD approaches, is thus expected to provide empirical estimates close to the local N_e under isolation. In contrast, such estimates are poor indicators of equilibrium N_{eIRx} at low migration rates. In fact, local N_{eVRx} at equilibrium is never even close to local N_{eIRx} for any value of m, and local N_{eLDRx} is only close at very high migration rates when the entire metapopulation is panmictic or nearly so.

The time required for reaching migration-drift equilibrium (Figure 3) can be very long at low migration rates. Thus, for $m' = 0.002$ (one immigrant per 10 generations), for example, it takes about 800 generations for N_{eIRx} to approach its approximate

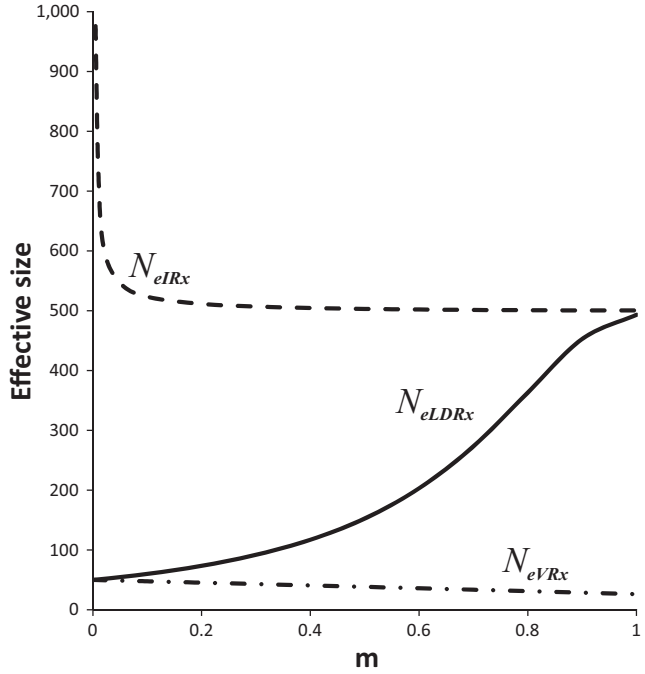

FIGURE 3 Equilibrium values for local inbreeding (N_{eIRx}), variance (N_{eVRx}), and linkage disequilibrium (N_{eLDRx}) effective size at different positive migration rates ($m > 0$). The values refer to an island model metapopulation with 10 ideal subpopulations of size $N_{ex} = N_{cx} = 50$ at migration-drift equilibrium. Note that the equilibrium condition implies that the curve for local N_{eIRx} coincides with that for the eigenvalue effective size (N_{eE}), which reflects the global inbreeding effective size (N_{eIMeta}) at equilibrium

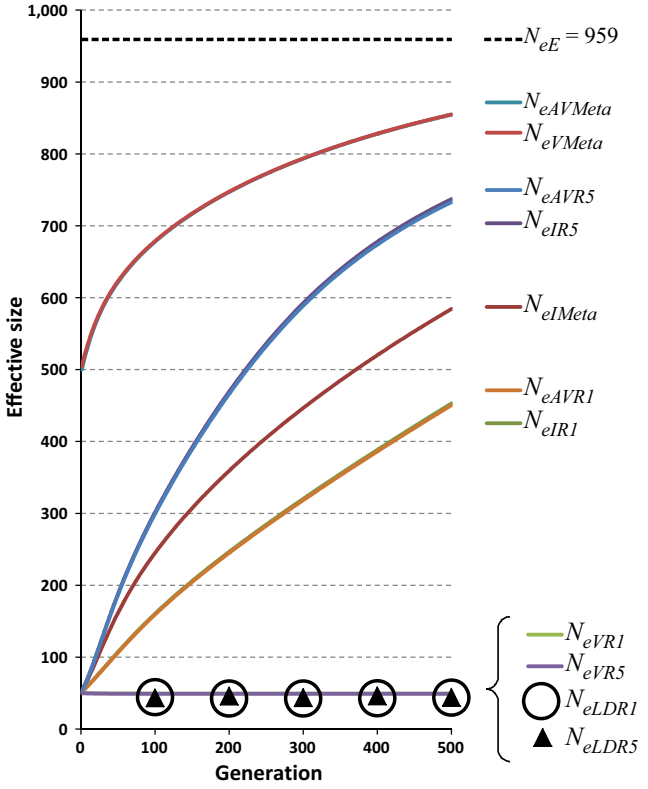

FIGURE 4 Global (*Meta*) and realized local (*Rx*) effective population sizes over 500 generations in a metapopulation following a linear stepping stone pattern of migration. There are ten (10) ideal subpopulations of constant effective size $N_{ex} = N_{cx} = 50$, and in every generation each subpopulation receives on average a half (0.5) immigrant drawn at random from each of the neighbouring ones. Realized local effective size is only given for subpopulation one and five (ordering from left to right) as indicated after the specific N_e, but note that the symmetry of the linear model implies that pairwise identical realized local N_e are expected for subpopulations 1 and 10, 2 and 9, etc. Rings and triangles represent simulated values at particular points in time. The eigenvalue effective size is $N_{eE} = 959$. Initial inbreeding and kinship is zero (0) within and between all subpopulations. Note that the scale of the y-axis differs from that in Figures 1 and 2. See Figure 1 for details on the different N_e

equilibrium value of $N_{eIRx} = N_{eIMeta} = N_{eE} = 1{,}590$ in the present metapopulation ($s = 10$, $N_{ex} = 50$), whereas N_{eVRx} and N_{eLDRx} will remain close their starting value of $N_{ex} = 50$ during the entire process. Further, the high values of N_{eIRx} at low migration rates should not be misinterpreted as suggesting complete or near isolation as an adequate strategy for genetic management of subdivided populations. The reason is that local inbreeding easily accumulates to unsatisfactorily high levels when migration is low. In the present example with $m' = 0.002$, for instance, the $N_{eIRx} = 500$ criterion will be met in generation $t \approx 275$. At this time, however, local inbreeding has increased to $f > 0.75$, a value that would most likely be considered unacceptably high in the context of genetic conservation (see Laikre et al., 2016 and below).

3.3 | Linear stepping stone model

We finally consider an ideal linear stepping stone model with the same basic demographic characteristics as the ones above, i.e., with $s = 10$ ideal subpopulations sized $N_{ex} = N_{cx} = 50$, which are now arranged in a line and numbered from left to right (Figure 4). Migration only occurs between neighboring subpopulations, and in every generation each subpopulation receives on average one half (0.5) immigrant from each neighbor. Thus, there is an average of one immigrant per generation into subpopulations 2–9 (as in the island model of Figure 1), whereas those at the ends (1 and 10) only get 0.5 immigrants. Due to this migration pattern the approach to equilibrium is much slower than for an island model with similar migration rates (Figure 4). The eigenvalue effective size is $N_{eE} = 959$, and all the local effective sizes expected to approach N_{eE} are still far from this value after 500 generations, particularly those for the "end" populations (1 and 10).

As for the island models, the realized local variance effective sizes in Figure 4 remain just under their initial value of $N_{ex} = 50$, and in generation $t = 500$ we have $N_{eVR1} = 49.3$ and $N_{eVR5} = 49.0$. The simulated values for the realized local N_{eLD} for subpopulations 1 and 5 vary in the range $N_{eLDR1,5} = 42$–46. Clearly, the tendency of realized local N_{eV} and N_{eLD} to follow trajectories that are strikingly different from those of the realized local N_{eI} and N_{eAV} persists also under the linear stepping stone model, which represents an extreme

relative to the island model with respect to connectivity (Allendorf et al., 2013; Kimura & Weiss, 1964).

4 | DISCUSSION

Applying recently developed theory on the genetic dynamics of metapopulations we have examined how different types of effective size change under the approach to migration-drift equilibrium, for the metapopulation as a whole and locally for each subpopulation. We have focused on N_{eI} and N_{eAV} relevant to the 50/500 conservation rule, and on N_{eV} and N_{eLD} that are frequently estimated from empirical data by commonly applied software. Two causes of genetic change have been considered, i.e., migration and local genetic drift within subpopulations. Our results can be summarized as follows.

1. In subdivided populations both the local and global inbreeding (N_{eI}) and additive genetic variance (N_{eAV}) effective sizes generally differ considerably from the local variance (N_{eV}) and linkage disequilibrium equilibrium (N_{eLD}) effective sizes. These discrepancies reflect true (parametric) differences between various types of N_e, and the bias can be indefinitely large. This is our most important finding because it implies that contemporary rates of inbreeding and/or loss of additive genetic variation are not assessed with commonly applied estimation tools.
2. The four types of N_e considered display quite different dynamics that is strongly dependent on migration model, migration rate, and the degree of deviation from equilibrium conditions, and the patterns are different for the metapopulation and the subpopulations. For instance, additive genetic variance (N_{eAV}) and variance (N_{eV}) effective size for the metapopulation follow the same trajectories in all the examples considered here, whereas their local equivalents do not (cf. $N_{eAVMeta}$, N_{eVMeta}, N_{eAVRx}, N_{eVRx} in Figures 1, 2 and 4). In contrast, local effective sizes reflecting actual rates of inbreeding and loss of additive variance exhibit similar trajectories, while those for the metapopulation as a whole differ radically (cf. N_{eIRx}, N_{eAVRx}, N_{eIMeta}, $N_{eAVMeta}$ in Figures 1, 2 and 4).
3. For an island model with high migration rates (say, m > ~0.85) the equilibrium values for local realized N_{eLDRx} and N_{eIRx} are similar, implying that estimators based on linkage equilibrium can assess the rate of inbreeding under nearly panmictic conditions (cf. Figure 3). In contrast, the variance effective size remains a poor predictor of contemporary inbreeding rate as long as the population is not completely isolated.
4. The different trajectories for the various forms of local N_e occur already at the small migration rate of one migrant per generation.

The difference between the behaviours of the global forms of variance and additive genetic variance effective sizes (N_{eVMeta} and $N_{eAVMeta}$) on one hand, and that of the global inbreeding effective size (N_{eIMeta}) on the other, is inherent to their definitions. N_{eVMeta} and $N_{eAVMeta}$ both relate to average allele frequencies of the metapopulation as a whole, quantities that change much more slowly than their counterparts in a local population, and this slow change is reflected in a "large" effective size. In contrast, N_{eIMeta} reflects the average change of individual inbreeding, thus necessarily relating to a process characterizing the dynamics within local subpopulations rather than of the metapopulation as a whole, which results in a "small" N_e.

It is important to note that the difference between the trajectories for the N_{eIRx} and N_{eAVRx} on one hand, and those for N_{eVRx} and N_{eLDRx} on the other, occurs already at quite small migration rates when estimates of local N_e obtained through the LD and temporal approaches are thought to be only marginally affected by migration. Waples and England (2011), for example, considered an ideal island model and concluded that LD estimates accurately reflect local (subpopulation) effective size unless m'> 0.05–0.10, and Ryman, Allendorf, Jorde, Laikre, and Hössjer (2014) arrived at a similar conclusion for the temporal method when using the same model. Our present island model example for one migrant per generation (Figure 1; m' = 0.02) thus represents a situation that should be considered "safe" for both methods. While this is true for N_{eVRx} and N_{eLDRx}, their trajectories are dramatically different from those for N_{eIRx} and N_{eAVRx} already at this small migration rate. Local realized N_{eV} and N_{eLD} are only slightly influenced by immigration at m' = 0.02, whereas the opposite is true for N_{eI} and N_{eAV}, implying that estimates of realized local N_{eV} or N_{eLD} are typically poor indicators of contemporary rates of inbreeding and loss of additive variation even at small migration rates.

Increasing migration to 10 individuals per generation (m' = 0.20) reveals a pattern that is qualitatively quite similar to that of m' = 0.02 (Figure 2 vs. Figure 1). The major difference is that the higher migration results in a faster approach to equilibrium conditions. Further, the trajectories for the local forms of variance and linkage disequilibrium effective size (N_{eVRx} and N_{eLDRx}) level out at values that are more distant from their starting point at N_e = 50 than at the lower migration rate. The local variance effective size is N_{eVRx} = 44.5 in generation t = 50 (compared to N_{eVRx} = 49.0 in Figure 1 where m' = 0.02), and this reduction is in agreement with the observations of Ryman et al. (2014). Using an ideal island model, but a somewhat different analytical approach, those authors showed that N_{eVRx} is expected to decrease from its initial value of 50 to $N_{eVRx} \approx N_{ex}/2$ as migration increases from m = 0 to the limiting value of m = 1. The reason for this low value of N_{eVRx} under panmixia (m = 1), is that the allele frequency change within a subpopulation is not only affected by local genetic drift, but also by migration from the other subpopulations which all have different allele frequencies.

Similarly for N_{eLDRx}, the expected local equilibrium value has now increased from N_{eLDRx} = 51.9 (at m' = 0.02; Figure 1) to N_{eLDRx} = 76.0 (at m' = 0.20; Figure 2). This increase is in line with simulation results of Waples and England (2011). Using an ideal island model they found that estimates of N_{eLDRx} tend to converge on the global effective size as migration increases towards m = 1. We also observe this and note that for an island model $N_{eIRx} = N_{eIMeta}$, and that $N_{eLDRx} \approx N_{eIRx}$ at equilibrium when m = 1 and (Figures 1, 2 and 3).

In all our models we have assumed that initial inbreeding and kinship is zero (0) within and between populations. Other initial conditions will change the values of N_{eI}, N_{eV}, N_{eAV}, and N_{eLD} (Hössjer et al., 2016), but this will not affect our main conclusion that these effective sizes are radically different in subdivided populations.

4.1 | Mutation and selection

The question arises how much other forces of genetic change, such as mutation (Durrett, 2008; Ewens, 1989) and selection, influence N_e. Germline mutations happen so rarely that they are typically not important for N_{eI} and short term protection of species. For N_{eAV} it does not seem justified to include mutation either, since mutation is already included as a factor that counteracts decreased genetic variance (Franklin, 1980). Selection can be of great importance to account for in the expression for the realized effective size when a particular gene or some other chromosomal region is of interest. On the other hand, when the whole genome of an organism is studied, the traditional view is that most regions will exhibit selectively neutral, or close to neutral, variation (Kimura, 1983; Ohta, 1973; Wang & Whitlock, 2003). This view has recently been challenged based on studies of genetic variation within the *Drosophila* genome, as well as comparative analyses with the genomes of related species (Charlesworth, 2012; Sella, Petrov, Przeworski, & Andolfatto, 2009). These results suggest that sometimes it may be valuable to include the impact of selection into our definitions of realized effective size, when sufficient information on the type, direction, and intensity of selection is available. The reason is that the rate of genetic drift will increase, and hence the effective size will decrease, in regions of the genome that are linked to non-neutral loci. This reduction of effective size is most common in regions of low recombination rate, when either directional (positive) selection occurs and the neutral, linked loci experience a hitchhiking effect, or when purifying (negative) selection occurs, and the neutral, linked loci experience background selection (Hudson & Kaplan, 1995; Kaplan, Hudson, & Langley, 1989). In diploid populations, the importance of these effects will not only depend on the fitness of single mutations, but rather on the fitness of genotypes. For instance, whereas deleterious mutations with a dominance effect will be removed rather quickly from the population, deleterious recessives may persist for a much longer time, with a different impact on the realized effective size,.

4.2 | Coalescence N_e and coalescence based methods

We have not included the coalescence effective size N_{eCo} in our numerical illustrations. There are several reasons for this. First, the original, mathematically elegant definition of N_{eCo} requires convergence of an ancestral tree towards Kingman's coalescent (Nordborg & Krone, 2002; Sjödin et al., 2005; Wakeley & Sargsyan, 2009) for any number of ancestral lines. This definition is quite restrictive, and therefore N_{eCo} rarely exists for subdivided populations unless the system is in equilibrium and the migration rate is large (Hössjer, 2011). Second, whenever N_{eCo} exists it equals N_{eE} (Hössjer, 2015), an effective size we already included in our numerical examples. Third, it is true that a weaker notion of N_{eCo} (the so called nucleotide diversity effective size) can be defined for pairs of ancestral lineages, even for populations of varying size (Durrett, 2008; Ewens, 1989). However, this more general type of coalescence effective size is closely related to a weighted harmonic average of N_{eGD} (or haploid N_{eI}) over different time horizons for haploid populations, or a weighted harmonic average of N_{eI} over time for diploid organisms, when the population starts from a level with no inbreeding and the population size is constant (see Hössjer et al., 2014,2015, and references therein).

In substructured populations the rate of coalescence between lineages that start from two gene copies in the present population will change through time because of migration that will result in ancestral lineages diffusing away from each other into different subpopulations as they trace back over time (Kelleher, Etheridge, Véber, & Barton, 2016; Mazet, Rodríguez, Grusea, Boitard, & Chikhi, 2016). Analytical coalescence based approaches have recently been developed, with the purpose of estimating historical effective sizes which are closely related to N_{eI} (Li & Durbin, 2011; Rasmussen, Hubisz, Gronau, & Siepel, 2014; Sheehan, Harris, & Song, 2013). These methods are typically applied to longer periods back in time, in order to fit the history of humans and other species. They have also been used to determine both historical and relatively recent genetic bottlenecks (Dussex, von Seth, Robertson, & Dalén, 2018). Clearly, the genetic history of populations is a concern in conservation since it has shaped present day levels of inbreeding and amount of additive genetic variance. However, in this paper we have not focused on those aspects of N_{eI} since our aim is to relate expected contemporary rates of inbreeding and loss of additive genetic variation to the N_e quantities estimated from assessing variance in allele frequencies and linkage disequilibrium.

4.3 | Conservation biology implications

Current estimation procedures for N_e typically strive at assessing effective size in isolation rather than realized effective size (e.g., Gilbert & Whitlock, 2015). The reason for the focus on N_e in isolation is not clear, but it may reflect a notion that N_{eV}, for example, can be reliably used as a substitute for N_{eI}. As we show, however, this is not correct for subdivided populations, and the error of the variance and linkage disequilibrium realized effective sizes, compared to the more relevant realized effective sizes which relate to contemporary rates of inbreeding or loss of additive genetic variation, may thus be immeasurably large. For an ideal infinite island model at equilibrium, for example, the effective sizes most relevant to conservation are infinitely large ($N_{eIRx} = N_{eAVRx} = N_{eE} = \infty$) regardless of the size of the subpopulations, whereas the quantity estimated is expected to be, depending on the migration rate, in the range $N_{ex}/2$ to N_{ex} for the

temporal method (Ryman et al., 2014) and N_{ex} to ∞ for the LD approach (Waples & England, 2011; this paper).

The issue of defining long-term conservation genetic goals relating to metapopulations has not yet been extensively dealt with in conservation research. An implicit suggestion has been that the same rule of thumb should apply for a subdivided population as for a single, isolated one, i.e., $N_{eIMeta} \geq 500$ should reflect long-term viability for the metapopulation as a whole (Hansen, Andersen, Aspi, & Fredrickson, 2011; Jamieson & Allendorf, 2012; Laikre, Jansson, Allendorf, Jakobsson, & Ryman, 2013). In a more detailed analysis of this issue Laikre et al. (2016) concluded that $N_{eIMeta} \geq 500$ cannot be the only focus for long-term genetic viability of a metapopulation. Rather, the inbreeding rates within the separate subpopulations must also be considered. They proposed that the conservation genetic target for metapopulations to reflect long-term genetic viability should imply that the rate of inbreeding in the system as a whole, as well as in the separate subpopulations, should not exceed $\Delta f = 0.001$ (as for an N_{eI} of 500). Thus, for long-term conservation they suggested that (a) metapopulation effective size is $N_{eIMeta} \geq 500$, and (b) realized inbreeding effective size of each subpopulation equals or exceeds 500 ($N_{eIRx} \geq 500$).

Applying the above line of reasoning from Laikre et al. (2016) all metapopulations and their subpopulations discussed in this paper would be considered genetically "safe", meeting the long-term goal of $N_e > 500$, before migration-drift equilibrium has been attained. This happens when the N_{eIRx} of the smallest subpopulation exceeds 500, which occurs in generation $t = 78$, $t = 13$, and $t = 577$ for Figures 1, 2 and 4, respectively.

In contrast, estimates obtained through the temporal method (N_{eVRx}) would, even at migration-drift equilibrium, be expected to vary around $N_{ex} = 50$ or less, many of them not even meeting the short-term conservation criterion of $N_e \geq 50$. The LD-method is expected to yield similar estimates at the lower migration rate of one individual per generation ($m' = 0.02$; Figures 1 and 4), and somewhat higher ones at $m' = 0.20$ ($N_{eLDRx} = 76.0$; Figure 2). In no case, however, would estimates be expected that are even close to signalling genetic safety (i.e., $N_{eIRx} \geq 500$). Rather, they would suggest some form of remedial action to reduce the inbreeding rate.

Estimates of realized local N_{eLD} tend to converge to global N_e as migration increases towards the limiting value of $m = 1$ (Figure 3, Waples & England, 2011; Supporting Information Appendix S1). Estimates of realized local N_e using the LD-method are thus expected to converge on the "right" value for N_{eIRx} when sampling from a metapopulation that is essentially panmictic. In contrast, realized local N_{eV} is expected to decrease towards $N_{ex}/2$ as m approaches $m = 1$ (Ryman et al., 2014). Such a difference between estimates from the LD and temporal methods ($N_{eLDRx} > N_{eVRx}$) could in some empirical situations hint that the targeted local population is under migration, potentially indicating that the true N_{eIRx} is (much?) larger than suggested by the empirical estimates. Differences of this kind should currently be evaluated with caution, though. For example, our ongoing work suggests that, under less ideal conditions than used in this study, the realized N_{eV} of a local population under migration may also be influenced by the census size (N_c). Under such circumstances the outcomes obtained from different estimators could be more difficult to interpret.

4.4 | Estimating effective size relevant to the 50/500 rule

Should N_e-estimation using the temporal or LD-approaches be discouraged? We see no reason for this, but it is crucial that such estimates are appropriately interpreted. Key issues are (a) whether the focal population receives immigrants or not, and (b) what form of effective size the investigator intends to estimate. In an isolated population (of Wright-Fisher type) of constant size all N_e are identical and can be used as proxies for the others. Under such circumstances any reasonably accurate estimator can be applied (c.f., Gilbert & Whitlock, 2015; Wang, Santiago, & Caballero, 2016). As we show, however, even minor immigration rates can result in a large difference between the form of N_e that is actually estimated and the one meant to be targeted. For the population depicted in Figure 1, for example, the migration rate ($m' = 0.02$) is so small that it is expected to only marginally affect the estimates of N_{eVRx} and N_{eLDRx} if applying the temporal and LD-approaches, respectively. Both estimates, however, are expected to differ dramatically from e.g., N_{eIRx} over nearly the entire period of approach to equilibrium, and by no means do they reflect the actual, contemporary rates of inbreeding or loss of additive genetic variation.

Further, although many papers on effective size stress the conservation perspective, there can be situations where the investigator is primarily interested in other forms of effective size such as N_{eGD}, N_{eLD}, N_{eV}, or N_{eCo} rather than N_{eI} and N_{eAV}. In such cases an estimator should, of course, be selected that matches the targeted form of N_e.

In the context of conservation and the 50/500 rule it seems reasonable to suggest a change of estimation approaches into ones that target N_{eI} and N_{eAV} rather than N_{eV} or N_{eLD}. We are aware of no method that assesses N_{eAV} directly, but pedigree data can be used for this purpose (Lynch & Walsh, 1998). Coalescence based methods can be used to estimate N_{eI} of the distant past (see above), whereas procedures based on multilocus heterozygote excess and sibship frequency are often mentioned as estimators targeting the inbreeding effective size of the present. Here, the heterozygote excess method is generally considered to show low precision and accuracy, whereas the performance of the sibship frequency method appears more promising (e.g., Gilbert & Whitlock, 2015; Luikart et al., 2010; Wang, 2016; Wang et al., 2016). We are not aware, however, of any study aimed at assessing their bias and precision for specific estimation of N_{eIRx} when this quantity is affected by local drift as well as migration. Actually, direct estimation of contemporary N_{eI} is not a trivial task unless pedigree data is available, because it should be based on assessments of shared identity by descent. Here, hidden Markov models have been applied to estimate inbreeding coefficients (Leutenegger et al., 2003) and coancestry coefficients (Browning & Browning, 2011; Lynch & Ritland, 1999). Such approaches may represent promising candidates for

expansion of present procedures to ones that permit assessment of N_{el} and other related forms of effective size in populations under migration.

Further, since N_{el} is defined in terms of increased inbreeding over time, it also requires at least two temporally spaced samples for direct estimation. It is difficult to see how this can be accomplished using the increasingly popular one sample estimators. In the lack of direct estimates of N_{el}, how do we deal with current assessments of N_e from populations that are, or may be, affected by migration? Estimates of N_{eVRx} are most likely biased downwards relative to N_{elRx} (Hössjer et al., 2016; this paper), and the same seems to hold also for N_{eLDRx}. Existing estimates should therefore be interpreted with caution, and with an understanding that they most likely reflect a lower limit for N_{elRx}, and thus an upper limit for the contemporary rate of increased inbreeding. The basic notion that N_{elRx} has been underestimated is strongly supported if there is independent information suggesting that immigration occurs. In a next step it can be helpful to try to identify the metapopulation involved with respect to the number of subpopulations, their size and pattern for connectivity. Even a crude picture of the characteristics of this metapopulation may be helpful in modelling the expected magnitudes of various forms of N_e using approaches similar to the present one.

4.5 | Beyond effective size

An alternative strategy could be to reduce the present focus on N_{el} and rates of inbreeding and rather concentrate on actual levels of inbreeding. Until recently, this has not been possible for natural populations, but next-generation sequencing approaches provide interesting openings. We have already mentioned hidden Markov models. Similarly, Kardos et al. (2018), for example, measured inbreeding in Scandinavian wolves from "identical-by-descent" chromosome segments (runs of homozygosity), and also found that these estimates correlated surprisingly well with pedigree data and with estimates obtained from 500 single nucleotide polymorphisms (SNPs). Focusing on inbreeding rather than effective size could also help modelling in some situations. For instance, N_{el} is only defined for populations where inbreeding increases, and cannot be used to properly describe genetic changes following immigration that reduces inbreeding for longer or shorter periods of time (cf. Hössjer et al., 2016; Laikre et al., 2016). Similarly, such a focus could aid in constructing more fine-tuned conservation strategies that also consider contemporary levels of inbreeding and not only the expected increase reflected by effective size. For instance, the goal of such a strategy could be to keep the inbreeding coefficient below a predefined threshold value over some time horizon.

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DATA ACCESSIBILITY

This study is theoretical and not based on empirical data. The software used are referenced and freely available. Analytical expressions have been published previously, except for those derived in the Supporting Information Appendix S1. Example scripts used to generate simulated genotypic distributions using the EASYPOP software (Balloux, 2001) are also provided as Supporting Information Appendix S2.

CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

The idea behind this paper developed through recurrent discussions among the authors on problems relating to estimation of effective population size and on the issue of defining conservation genetic goals for metapopulations within a project lead by L.L. Calculations were completed by N.R. who also conducted the computer simulations and wrote the first version of the manuscript with input from both coauthors. O.H. derived the mathematical expressions, wrote the first version of the Supporting Information, and contributed theoretical input throughout the project. All the authors participated in discussions that developed the work, and they actively contributed to the writing process that was led by N.R.

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