# A method to estimate effective population size from linkage disequilibrium when generations overlap

Luis Alberto García Cortés[1], Frédéric Austerlitz[2], and M. Ángeles R. de Cara[2,3,4]

[1] Departameto de Mejora Genética Animal, INIA, Ctra. de La Coruña, km 7,5, 28040 Madrid, Spain

[2]UMR 7206 EcoAnthropologie et Ethnobiologie, CNRS/MNHN/Université Paris Diderot, Musée de l'Homme, Paris, France

[3]Centro de Investigação em Biodiversidade e Recursos Genéticos (CIBIO-UP), InBIO, Universidade do Porto, Porto, Portugal

[4]Corresponding author. angeles.decara@cibio.up.pt

**Abstract**

Effective population size ($N_e$) is a key parameter in evolutionary and conservation studies. It characterises the number of unique or distinct individuals in a population, and can be used to establish management programmes. Several methods have been developed to estimate this parameter. Currently, for studies with one sample in time, the simplest methods are based on linkage disequilibrium. These methods rely on simple models, and biases have been shown when populations deviate from the assumptions made in those models. This occurs in particular when populations are age-structured or have overlapping generations. Recently, several methods have been developed to correct such biases. Here, we develop analytical equations to predict linkage disequilibrium within age groups, and use such results to infer cohort size from

samples of newborn individuals. We can in turn use these equations to estimate $N_e$ accurately for a variety of species. Furthermore, using publicly available data, we apply our method to the white-crowned sparrow (*Zonotrichia leucophrys*).

**Introduction** Linkage disequilibrium is the association between alleles at different loci within or between chromosomes. It depends on allelic frequencies, recombination, mutation and effective population size ($N_e$, Hill & Robertson 1968). Thus, it can be used to make inferences about effective size, a crucial parameter in evolutionary and conservation studies. It is commonly considered that populations with $N_e$ smaller than 50 are endangered in the short-term and that conservation programmes are often necessary for such populations (Franklin 1980). Most analytical predictions on linkage disequilibrium (LD) have been obtained in populations with non-overlapping generations, and such simple models have allowed a whole body of research aiming at estimating $N_e$ from LD, starting with the pioneering work of Sved (1971).
Sved followed the work of Hill & Robertson (1968) which showed how alleles at different positions in the genome tend to become associated in finite populations. He studied LD between two biallelic loci and established that it is equivalent to studying identity by descent (IBD). By analysing the temporal dynamics of the allelic frequencies at the two loci ignoring mutation, he derived an analytical formula establishing the relationship between $N_e$ and LD. In later work, Sved & Feldman (1973) took into account sampling with replacement, which resulted in a slight modification to Sved's (1971) formula. Weir & Hill (1980) and Hill (1981) also developed methods to infer $N_e$ from LD measures, taking into account sample size and mating system.
This body of work was not much used for a long time on real data, with a few exceptions in *Drosophila* and human data (Loukas *et al* 1979; Malpica & Briscoe 1982; Piazza *et al* 1985).
With the advent of dense genotyping data, in particular in humans and livestock, and the work of Hayes *et al* (2003) who developed methods for inferring current and ancestral $N_e$, such estimates have become common to the point where they constitute the prevailing method nowadays. In parallel to theoretical work on the relationship between $N_e$ and LD, there has been a growing body of research showing the effects of population and age structure on estimates of $N_e$. This started

with the work of Felsenstein (1971) who derived equations for $N_e$ given a known age structure and Hill (1972) showed that $N_e$ in such populations is equal to that of a population with no overlapping population with the same variance in lifetime family size. Orive (1993) used a coalescent approach to generalise to populations with overlapping generations and with sexual or asexual reproduction. These works have mostly been used to characterise $N_e$ in populations by studying their age structure, without making use of information on linkage disequilibrium. Only recently methods using both LD and data on age structure have been developed to improve estimates of $N_e$ (Robinson & Moyer 2013; Waples *et al* 2014). These works have shown that using only LD to infer $N_e$ in populations with overlapping generations can result in biased estimates, and Waples *et al* (2014) introduced heuristic modifications to correct such biases.

Here we develop analytical methods to account for age structure in the estimates of $N_e$ from linkage disequilibrium. We start by deriving equations for linkage disequilibrium measured via $r^2$ (Hill & Robertson 1968) in a scenario consisting in a population with several cohorts of varying fertility and survival rates. We use the equivalence between $r^2$ and identity by descent to relate these genetic measures to $N_e$. By inverting the system of equations derived, we can then estimate the cohort size and the effective population size from genetic data. Lastly, we apply our method to publicly available data from the white-crowned sparrow (*Zonotrichia leucophrys*, Lipshutz *et al* 2017).

## Methods and material

Sved's (1971) method defines $Q$ as the probability of identity by descent (IBD) of two individuals at locus B given that locus A is also IBD. This probability $Q$ is also called the joint homozygosity and represents the probability of no crossing over between loci A and B since the common ancestor. Sved (1971) showed that $E\left(r^2\right) = Q$, where $r^2 = D^2/\left(p_A p_a p_B p_b\right)$ is a measure of linkage disequilibrium between loci A and B, and derived the following probability of joint homozygosity in the next generation, $Q'$, as

$$Q' = \frac{1}{2N}(1-c)^2 + \left(1 - \frac{1}{2N}\right) Q\,(1-c)^2\,, \tag{1}$$

where $c$ is the recombination fraction between loci $A$ and $B$ and $N$ is the effective population size. The recombination fraction is obtained as $c = 0.5\left(1 - e^{-2d}\right)$ where $d$ is the map distance (Haldane 1919).

This equation was later revised by Sved & Feldman (1973) as

$$Q^{'} = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) Q\,(1 - c)^2\,, \tag{2}$$

to allow sampling with replacement. The details of this equation were clarified recently by Sved (2019), where he replaced $Q$ with $L$, which denotes the linked identity by descent (LIBD), $L$, which considers sampling with replacement. Some useful details can be found there concerning the derivation of eq. (2) and the difference with eq. (1). Thus,

$$L^{'} = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) L\,(1 - c)^2\,. \tag{3}$$

It must noted that $Q$ and $L$, while similar in concept, are not identical. $Q$ is the probability of joint IBD without replacement while $L$ is the probability of joint IBD, but allowing for replacement. Earlier work has shown that $L$, as derived in Sved & Feldman (1973), provides better estimations of $E\left[r^2\right]$ (Garcia-Cortes *et al* 2019).

Equations (1), (2) and (3) were developed for populations with non overlapping generations. Nevertheless, many species have overlapping generations and this cannot be ignored when evaluating the intensity of genetic drift (Felsenstein 1971), or calculating the equilibrium between genetic drift and recombination. Most importantly, researchers have been using estimates of $N_e$ based on these equations without much caution about potential pitfalls (Robinson & Moyer 2013; Waples *et al* 2014).

In populations with age structure, following Felsenstein (1971), the new cohort is the progeny of some parental age groups with $N_i = s_i N_0$ individuals, where $N_0$ is the number of newborns and $s_i$ is probability of survival to age $i$. Every newborn has a probability $p_i$ of coming from a parent of age $i$, and assuming that $N_0$ remains constant, $\sum_i p_i = 1$.

Following the notation for $Q$ of Sved (1971), we denote $Q_{ij}$ as the probability of no crossover from a common ancestor of a pair of gametes from age groups $i$ and $j$ and $Q_{ii}$ is the probability of no crossover between two different gametes of age group $i$, *i.e.* two gametes sampled without replacement. Then, at a given time interval,

$$\mathbf{Q} = \left[\begin{array}{c|cccc} Q_{00} & Q_{01} & Q_{02} & Q_{03} & \cdots \\ \hline Q_{10} & Q_{11} & Q_{12} & Q_{13} & \cdots \\ Q_{20} & Q_{21} & Q_{22} & Q_{23} & \cdots \\ Q_{30} & Q_{31} & Q_{32} & Q_{33} & \cdots \\ \vdots & \vdots & \vdots & \vdots & \ddots \end{array}\right]$$

where 0 is the group of newborns. The definition of matrix $\mathbf{Q}$ here mimics the definition of the heterozygosity matrix in Felsenstein (1971).

We denote $Q'_{ij}$ as the elements of $\mathbf{Q}$ at the next unit time. Every generation or time unit the $Q$ at each cohort can be written in terms of contributions from other $Q$s at other cohorts, and analogously for between cohorts $Q_{ij}$. In fig. 1, we show the five possible origins of a pair of random gametes in a population with two parental age groups. This can be easily extended for $\left(n^2 + 3n\right)/2$ contributions with $n$ parental groups. The recursive formula to obtain $Q'_{00}$, i.e. $Q_{00}$ at the next time unit, given the elements of $\mathbf{Q}$ can be obtained adding all contributions on fig. 1. This can be generalised to any number of age groups such that

$$\begin{aligned} Q'_{00} &= (1-c)^2 \sum_i p_i^2 \left[\frac{1}{2N_i} + \left(1 - \frac{1}{2N_i}\right) Q_{ii}\right] \\ &+ 2\,(1-c)^2 \sum_i \sum_{j<i} p_i p_j Q_{ij}, \end{aligned} \tag{4}$$

where the term $(1-c)^2 \sum_i p_i^2 \left[\frac{1}{2N_i} + \left(1 - \frac{1}{2N_i}\right) Q_{ii}\right]$ corresponds to the sum of the contribution of cases (a), (b), (d) and (e) in fig. 1 while the term $2\,(1-c)^2 \sum_i \sum_{j<i} p_i p_j Q_{ij}$ corresponds to case (c) extended for an arbitrary number of cohorts. In order to derive the probabilities of each case, and following Sved (1971), the term $(1-c)^2$ accounts for no crossover in either maternal or paternal

meiosis, while $p_i^2$ or $p_i p_j$ is the probability of a pair of gametes to come from the same or different age groups. Lastly, $\frac{1}{2N_i}$ or $\left(1 - \frac{1}{2N_i}\right)$ is the probability of ancestral gametes are identical or not, respectively.

Fig. 2 shows the contributions of the three possible cases for both $Q'_{01}$ and $Q'_{02}$ (or $n + 1$ contributions for $n$ age groups). Adding all contributions for an arbitrary number of age groups, the recursive formula to obtain each $Q'_{0i}$ is as follows

$$\begin{aligned} Q'_{0i} &= (1-c)\, p_i \left[ \frac{1}{2N_i} + \left(1 - \frac{1}{2N_i}\right) Q_{ii} \right] \\ &+ (1-c) \sum_{j \neq i} p_j Q_{ij}. \end{aligned} \tag{5}$$

Given that individuals from cohort $i$ will move onto cohort $i + 1$ in the next time unit, the joint homozygosity between survivors of cohort $i$ and cohort $j$ in a time unit remains the same in the next time unit. Therefore, the remaining elements in $\mathbf{Q}'$ are

$$Q'_{ij} = Q_{i-1,j-1}, \tag{6}$$

and

$$Q'_{ii} = Q_{i-1,i-1}. \tag{7}$$

The recursive formulae given by eq. (4) to eq. (7) reach an equilibrium $\mathbf{Q}^{eq}$ where drift and recombination balance out. At equilibrium $Q'_{ij} = Q_{ij}$ and, considering eq. (6) and eq. (7), it follows that $Q_{00} = Q_{11} = Q_{22}$, $Q_{01} = Q_{12} = Q_{23}$, $Q_{02} = Q_{13}$ and so on. Thus, the matrix given by

$$\mathbf{Q}^{eq} = \left[ \begin{array}{c|cccc} Q^{eq}_{(00)} & Q^{eq}_{(01)} & Q^{eq}_{(02)} & Q^{eq}_{(03)} & \cdots \\ \hline Q^{eq}_{(01)} & Q^{eq}_{(00)} & Q^{eq}_{(01)} & Q^{eq}_{(02)} & \cdots \\ Q^{eq}_{(02)} & Q^{eq}_{(01)} & Q^{eq}_{(00)} & Q^{eq}_{(01)} & \cdots \\ Q^{eq}_{(03)} & Q^{eq}_{(02)} & Q^{eq}_{(01)} & Q^{eq}_{(00)} & \cdots \\ \vdots & \vdots & \vdots & \vdots & \ddots \end{array} \right]$$

and equations eq. (4) and eq. (5) can be solved using

$$\begin{bmatrix} \frac{1}{(1-c)^2} - \sum_i p_i^2(1-\frac{1}{2N_i}) & -\alpha_1 & -\alpha_2 & \dots \\ -p_1(1-\frac{1}{2N_1}) & \frac{1}{(1-c)} - \beta_{11} & -\beta_{12} & \dots \\ -p_2(1-\frac{1}{2N_2}) & -\beta_{21} & \frac{1}{(1-c)} - \beta_{22} & \dots \\ \dots & \dots & \dots & \dots \end{bmatrix} \begin{bmatrix} Q^{eq}_{(00)} \\ Q^{eq}_{(01)} \\ Q^{eq}_{(02)} \\ \dots \end{bmatrix} = \begin{bmatrix} \sum_i \frac{p_i^2}{2N_i} \\ \frac{p_1}{2N_1} \\ \frac{p_2}{2N_2} \\ \dots \end{bmatrix}, \tag{8}$$

where $\beta_{ik} = \sum_j \delta_{|i-j|,k} p_j$ and $\alpha_k = \sum_i \sum_j \delta_{|i-j|,k} p_i p_j$. The function $\delta_{i,j}$ is Kroenecker's delta, that is, $\delta_{i,i} = 1$ and $\delta_{i,j} = 0$ for $i \neq j$.

*Dynamics of* **L** *throughout generations*

Here, we derive the equations for $L$, the probability of linked identity by descent, at two positions, as defined by Sved & Feldman (1973) and Sved (2019). We define $L_{ij}$ as the LIBD of a pair of gametes from age groups $i$ and $j$ and $L_{ii}$ as the LIBD of two gametes of age group $i$, drawn at random with replacement. Then, at any given generation, $L$ is given by

$$\mathbf{L} = \left[\begin{array}{c|cccc} L_{00} & L_{01} & L_{02} & L_{03} & \cdots \\ \hline L_{10} & L_{11} & L_{12} & L_{13} & \cdots \\ L_{20} & L_{21} & L_{22} & L_{23} & \cdots \\ L_{30} & L_{31} & L_{32} & L_{33} & \cdots \\ \vdots & \vdots & \vdots & \vdots & \ddots \end{array}\right].$$

Unlike $Q$, $L$ represents the LIBD between two gametes with replacement. Considering this difference, and using the relationships derived in the appendix **A**, the relationship between $Q$ and $L$ is thus straightforward:

$$L_{ij} = Q_{ij}, \text{for } i \neq j, \tag{9}$$

and

$$L_{ii} = \frac{1}{2N_i} + \left(1 - \frac{1}{2N_i}\right) Q_{ii}. \tag{10}$$

To calculate $L'_{00}$, we replace eq. (9) and eq. (10) in eq. (4):

$$\begin{aligned} Q'_{00} &= (1-c)^2 \sum_i p_i^2 L_{ii} + 2\,(1-c)^2 \sum_i \sum_{j<i} p_i p_j L_{ij} \\ &= (1-c)^2 \sum_i \sum_j p_i p_j L_{ij}, \end{aligned}$$

and replacing in eq. (10), we obtain that

$$L'_{00} = \frac{1}{2N_0} + \left(1 - \frac{1}{2N_0}\right)(1-c)^2 \sum_i \sum_j p_i p_j L_{ij}. \tag{11}$$

Similarly, to calculate $L'_{0i}$, we replace both eq. (9) and eq. (10) in eq. (5), obtaining

$$\begin{aligned} Q'_{0i} &= (1-c)\, p_i L_{ii} + (1-c) \sum_{j \neq i} p_j L_{ij} \\ &= (1-c) \sum_j p_j L_{ij}. \end{aligned}$$

We can use these results in eq. (9):

$$L'_{0i} = (1-c) \sum_j p_j L_{ij}. \tag{12}$$

Equations (11) and (12) can be derived from the probabilities for $Q$ shown in fig. (1) and fig. (2) but accounting for sampling with replacement. Both procedures are equivalent.

It then follows from eq. (6) and eq. (9) that

$$L'_{ij} = L_{i-1,j-1}. \tag{13}$$

In fact, sampling with or without replacement are equivalent when considering gametes from different age groups. Finally, to calculate $L'_{ii}$ we rewrite eq. (10) as

$$Q_{ii} = \frac{L_{ii} - \frac{1}{2N_i}}{1 - \frac{1}{2N_i}},$$

and replace it in eq. (7), which results in

$$L_{ii}^{'} = \frac{1}{2N_i} + \frac{1-\frac{1}{2N_i}}{1-\frac{1}{2N_{i-1}}}\left(L_{i-1,i-1} - \frac{1}{2N_{i-1}}\right). \tag{14}$$

Using all the above results, eq. (14) can be rewritten as

$$L_{ii} = \frac{1}{2N_i} + \frac{1-\frac{1}{2N_i}}{1-\frac{1}{2N_0}}\left(L_{00} - \frac{1}{2N_0}\right). \tag{15}$$

At equilibrium, $L_{ij}^{'} = L_{ij}$ and, following eq. (13), $L_{01} = L_{12} = L_{23}$, $L_{02} = L_{13}$, ... and so on. Thus,

$$\mathbf{L}_{eq} = \left[\begin{array}{c|cccc} L_{00}^{eq} & L_{01}^{eq} & L_{02}^{eq} & L_{03}^{eq} & \cdots \\ \hline L_{01}^{eq} & L_{11}^{eq} & L_{01}^{eq} & L_{02}^{eq} & \cdots \\ L_{02}^{eq} & L_{01}^{eq} & L_{22}^{eq} & L_{01}^{eq} & \cdots \\ L_{03}^{eq} & L_{02}^{eq} & L_{01}^{eq} & L_{33}^{eq} & \cdots \\ \vdots & \vdots & \vdots & \vdots & \ddots \end{array}\right]. \tag{16}$$

Therefore, at equilibrium

$$\begin{bmatrix} 1-\zeta_{11} & -\zeta_{12} & -\zeta_{13} & ... & 0 & -\tau_1 & 0 & 0 & ... \\ -\zeta_{21} & 1-\zeta_{22} & -\zeta_{23} & \cdots & 0 & 0 & -\tau_2 & 0 & \cdots \\ -\zeta_{31} & -\zeta_{32} & 1-\zeta_{33} & \cdots & 0 & 0 & 0 & -\tau_3 & \cdots \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots & \vdots & \vdots & \\ -\varphi_1 & -\varphi_2 & -\varphi_3 & \cdots & 1 & -\gamma_1 & -\gamma_2 & -\gamma_3 & \cdots \\ 0 & 0 & 0 & \cdots & -\xi_1 & 1 & 0 & 0 & \cdots \\ 0 & 0 & 0 & \cdots & 0 & -\xi_2 & 1 & 0 & \cdots \\ 0 & 0 & 0 & \cdots & 0 & 0 & -\xi_3 & 1 & \cdots \\ \vdots & \vdots & \vdots & & \vdots & \vdots & \vdots & \vdots & \ddots \end{bmatrix} \begin{bmatrix} L_{01}^{eq} \\ L_{02}^{eq} \\ L_{03}^{eq} \\ \vdots \\ L_{00}^{eq} \\ L_{11}^{eq} \\ L_{22}^{eq} \\ L_{33}^{eq} \\ \vdots \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ \vdots \\ \frac{1}{2N_0} \\ \varrho_1 \\ \varrho_2 \\ \varrho_3 \\ \vdots \end{bmatrix}, \tag{17}$$

where $\zeta_{ik} = (1-c)\beta_{ik}$, $\tau_i = (1-c)p_i$, $\varphi_k = \left(1-\frac{1}{2N_0}\right)(1-c)^2\,\alpha_k$, $\gamma_k = \left(1-\frac{1}{2N_0}\right)(1-c)^2\,p_k^2$,

$\xi_i = \frac{1-\frac{1}{2N_i}}{1-\frac{1}{2N_{i-1}}}$ and $\varrho_i = \frac{1}{2N_i} - \frac{\xi_i}{2N_{i-1}}$.

It is well known that sampling with replacement usually leads to equations simpler than those derived considering sampling without replacement. For this reason, eq. (11) and eq. (12) are simpler than eq. (4) and eq. (5). Nevertheless, the number of equations to solve for $L^{eq}$ is larger than the number of equations required to solve $Q^{eq}$, which accounts for differences between the diagonal elements of matrix (16).

*Estimation of $N_0$ from linkage disequilibrium*

As $E\left(r^2\right) = L$ (Sved & Feldman 1973), we can use the equations described in the previous section to estimate $N_0$ from observed values of linkage disequilibrium. We thus use eq. 11, eq. 12, eq. 13 and eq. 14, but considering $N_0$ as unknown and $L_{00}^{eq}$ as observed for a given value of recombination frequency.

The equations for $N_0$ are nonlinear but can be solved by iterating

$$2N_0 = \frac{1 + (1-c)^2 \sum_k (\alpha_k L_{0k}^{eq} - p_k^2 L_{kk}^{eq})}{L_{00}^{eq} + (1-c)^2 \sum_k (\alpha_k L_{0k}^{eq} - p_k^2 L_{kk}^{eq})}, \tag{18}$$

and the rest of identities in eq. (17), that is

$$L_{0i}^{eq} = \frac{\sum_{j \neq i} \zeta_{ij} L_{0j}^{eq} + \tau_i L_{ii}^{eq}}{1 - \zeta_{ii}}, \tag{19}$$

and

$$L_{ii}^{eq} = \varrho_i + \xi_i L_{i-1,i-1}^{eq}. \tag{20}$$

*Numerical analyses*

We compared our analytical results with simulated results. For this purpose, we followed these steps in our simulations:

1. We drew the number of newborn gametes coming from each group age ($n_i$) using multinomial deviates $n_1, n_2, ... \sim M\left(N_0, p_1, p_2, ...\right)$, where $p_i$ is the proportion of the $N_0$ newborn gametes that have been born from individuals from cohort $i$.

2. For each cohort $i$ then

   (a) We drew the number of recombined ($nrec_i$) or non-recombined ($norec_i$) gametes using Bernouilli deviates $nrec_i \sim B(n_i, c)$.

   (b) We drew the number of non-recombined progeny carrying each haplotype ($AB$,$ab$,$aB$ and $Ab$) following a multinomial distribution $M(norec_i, p_{AB}(i), p_{Ab}(i), p_{aB}(i), p_{ab}(i))$, where $p_{AB}(i)$,$p_{Ab}(i)$,$p_{aB}(i)$ and $p_{ab}(i)$ represent the haplotypic frequencies of cohort $i$.

   (c) We drew the number of recombined progeny carring each haplotype given the multinomial distribution $M(rec_i, p_A(i)P_B, p_A(i)P_b, p_a(i)P_B, p_a(i)P_b)$, where $p_A(i)$ and $p_a(i)$ are the allelic frequencies of cohort $i$. $P_B$ and $P_b$ represent the allelic frequencies of the whole population.

3. We calculated the haplotypic frequencies of newborn individuals by adding up gametes from steps 2b and 2c.

4. For each cohort $i$

   (a) We then calculated the number of individuals to be removed $died \sim B[N_{i-1}, (N_{i-1} - N_i)/N_{i-1}]$ using $N_i$ and $N_{i-1}$,

   (b) The number of individuals carrying each haplotype to be removed was then calculated following $M(died, p_{AB}(i-1), p_{Ab}(i-1), p_{aB}(i-1), p_{ab}(i-1))$, where $p$ are haplotypic frequencies at group age $i-1$

   (c) Individuals were eliminated to calculate the new haplotypic frequencies at each cohort $i$.

Recombined and non-recombined gametes at step 2a include gametes that have undergone an odd or even number of crossovers, respectively.

*Application to real data* The programs required to infer $N_0$ and $N_e$ from observed $r^2$ are available at `https://github.com/angelesdecara/OverlapNeLD`. We applied our method to available

data from white-crowned sparrow (*Zonotrichia leucophrys nuttalli*) (Lipshutz *et al* 2017), whose age structure has been described in earlier works (Baker *et al* 1981; Waples & Yokota 2007). Data from 169 individuals are available for 6419 SNPs obtained using genotyping-by-sequencing (GBS). They can be classified in three different groups, depending on whether they are ssp. nuttalli, ssp. pugetensis or admixed. We calculated $N_0$ and $N_e$ using the values for the fraction of a cohort that survives to a certain age, and the mean number of offspring produced per generation by individuals of each age group from Waples & Yokota (2007), separately for each subspecies. Linkage disequilibrium was estimated using the option `-geno-r2` of vcftools (Danecek *et al* 2011). Note that this measure of linkage disequilibrium is the correlation between allelic frequencies not taking into account gametic phases. Thus, it is an approximation to the gametic linkage disequilibrium. Furthermore, there is no chromosome scale reference genome for this species, and the number of data is small. We corrected this estimate of $r^2$ to account for sample size following Weir & Hill (1980); Waples (2006); Waples & Do (2008), by the harmonic sample size for each pair of loci (see Waples & Do 2008, for further details).
As there is no available linkage map, we had no information on the positions of each SNP. Thus, we assumed that $c = 0.5$, that is, that all SNPs were independent. Thus, the results here obtained are likely to be downward biased. We compared our results with those obtained using `Ne estimator` (Waples & Do 2008) based on LD using all informative SNPs, equally assuming that SNPs are unlinked.

## Results

We compare here the results obtained analytically with those obtained computationally using multinomial distributions, following the above algorithm. The population here studied consists of three cohorts or age groups.We have studied the three following scenarios:

1. All contributions are the same (i.e. all $N_i$'s take the same value equal to 1000 diploid individuals) and $p_1 = p_2 = p_3 = \frac{1}{3}$.

2. All cohorts have the same number of individuals (all $N_i = 1000$ diploids), but they

contribute differently to the number of newborns, due to different fertilities per age group, such that $p_1 = 0.3$, $p_2 = 0.5$ and $p_3 = 0.2$.

3. Cohorts have different sizes ($N_1 = 1000$, $N_2 = 1600$ and $N_3 = 500$) and contribute differently $p_1 = 0.3$, $p_2 = 0.5$ and $p_3 = 0.2$.

Fig. 3 shows analytical and simulated results for $L_{00}$, in black and green respectively, for a population with 3 cohorts and $N_0 = 1000$ diploid individuals. The difference between the estimated and the observed $r^2$ (that is, estimated from $L_{00}$ and measured using a multinomial approach) is larger as the contribution of one cohort increases as compared to the other cohorts, and furthermore, this difference increases with cohort age. In other words, the difference between estimated and observed when cohort 1 is the only one contributing is zero, but when cohort 3 is the most contributing this difference is larger than when cohort 2 is the most contributing.

As shown in tab. 1, we can see how linkage disequilibrium as measured by $r^2$ is in very good agreement with the results obtained for $L_{00}$, which takes into account sampling with replacement, while $Q_{00}$ differs noticeably. This is the case for the three scenarios, with the difference between $r^2$ and $Q_{00}$ increasing with increasing complexity. Scenario 3 shows the largest where differences between $r^2$ and $L_{00}$, but both measures are still in very good agreement, for a variety of recombination probabilities.

Estimates of $N_0$ from $L_{00}$ are shown in tab. 2, using either 100 or 500 pairs of loci to make estimates. In both cases, the results are for $N_0 = 1000$ diploid individuals and we can see how the inferred $N_0$ is mostly accurate within 10% deviation, with three exceptions for scenario 2.

Lastly, we have analysed the dependence on $c$ and $N_0$. In fig. 4, we can see how the inferred $N_0$ deviates from its true value of 1000 depending on the recombination fraction $c$. This deviation is stronger for tighter linkage, i.e. small $c$. In fig. 5, we can see how the inferred $N_0$ has a high accuracy under all scenarios for all recombination rates, with a few exceptions for scenario 2.

A detailed analysis of the estimated $N_0$ versus recombination frequency is shown in Fig. 4, where we can see the three scenarios studied. Overall, the larger the recombination frequency, the better the estimate of $N_0$.

Similar results are shown in Fig. 5, where we can see the dependency of the estimated $N$ with its true value for four recombination frequencies. The estimate provides a good result overall.

*Application to real data: white crowned sparrows* Using the life-history parameters from Waples & Yokota (2007) and our exact equations, we inferred $N_0$, and then, following Felsenstein (1971), we used this result to infer $N_e$. Results are shown in Table 3. There we can see that the inferred $N_e$ is rather low, which would indicate the need for a conservation programme. We believe this is partly due to the assumption of independent SNPs, and using a different value of $c$ would result in larger estimates of $N_e$.

The estimates for $N_e$ are considerably lower than those obtained by Lipshutz *et al* (2017) using fastsimcoal (Excoffier *et al* 2013), which uses the site frequency spectrum (SFS) to make demographic inferences. This discrepancy between estimates based on LD or those based on fastsimcoal using the SFS has been observed in other studies (Sovic *et al* 2019). The differences may be due to several factors, like the fact that Lipshutz *et al* (2017) assumed constant population sizes throughout long time-scales, and allowed for migration (Santiago *et al* 2020).

**Discussion** Following Sved (1971); Sved & Feldman (1973) and earlier work (Garcia-Cortes *et al* 2019), we have derived here exact equations for linkage disequilibrium in populations with overlapping generations. The results can then be used to estimate effective population size, a key parameter in evolutionary and conservation studies. The strength of our method is that we do not require any approximation, as it relies on an analytical approach.

Our goal here was partly to take into account the developments made by Sved & Feldman (1973); Sved (2019) so that sampling with replacement is considered. Sved (2019) emphasised the linked identity by descent ($L$), which is conceptually equivalent to what he called earlier (Sved 1971) joint identity by descent ($Q$). Both identities are related by equations (9) and (10).

Our results show that using the linked identity by descent provides better estimates, which is in agreement with our earlier work (Garcia-Cortes *et al* 2019). We have further compared our exact results with those obtained via simulations, using multinomial distributions, and have obtained a very good agreement between them.

Our method is reliable and behaves well even with a strong age-structure, as seen on Fig. 4 and Fig. 5. Based on Fig. 4, the best estimates are obtained for loosely linked or unlinked markers. The good agreement can also be observed in Fig. 3, where we can see the good agreement between the estimated and the simulated size. It seems that the older the most contributing cohort, the largest the differences between the estimated and the simulated size. Even so, the estimates are very good and mostly within 10% relative error.

We have developed equations to infer $N_e$ starting from $L_{00}$, that is, linkage disequilibrium within newborns. Using such measure, we can derive all linkage disequilibria within and between cohorts, which leads to the inference of $N_e$ by using the sets of equations( 17). Alternatively, we could take a random sample from the population to estimate overall linkage disequilibrium. In that case, we could infer $N_e$ combining this overall estimate of LD, which is the weighted sum of the linkage disequilibria between and within cohorts. This would add further complexity to the problem, as it would require equations( 17) together with this weighted average.

Finally, we have used our analytical results to infer $N_e$ in the white-crowned sparrow, for which genetic and demographic data were available (Baker *et al* 1981; Lipshutz *et al* 2017). The results shown in Tab. 3 are in line with our previous results. We had shown for non-overlapping generations, that the estimator of Weir & Hill (1980) were downward biased (Garcia-Cortes *et al* 2019). In Table 3 we observe a similar trend, where the results obtained with `Ne estimator`, based on the estimator developed by Weir & Hill (1980), are smaller than those obtained with our method. We have to emphasise that this dataset suffers from a small sample size, lack of linkage map and using an estimate of $r^2$, as gametic phases are unavailable. Obiously, our results are still limited by the assumptions here made, like constant age structure and constant population size. Still, this study shows the potential of our method to make estimates of effective population size in wild species using the available program at `http://github.com/angelesdecara/OverlapNeLD`.

**Acknowledgements** This work was funded by Ministerio de Economía y Competitividad grant number AGL2016-75942-R, by the LabEx BCDIV (ANR-10-LABX-0003-BCDiv), within the

framework of the program “Investing for the future” (ANR-11-IDEX-0004-02) and by the Agence Nationale de la Recherche project AGRHUM (ANR-14-CE02-0003).

*

## References

Baker M, Mewaldt LR, Stewart RM (1981) Demography of white-crowned sparrows (*zonotrichia leucophrys nuttalli*). *Ecology*, **62**, 636–644.

Danecek P, Auton A, Abecasis G, *et al* (2011) The variant call format and vcftools. *Bioinformatics*, **27**, 2156–2158.

Excoffier L, Dupanloup I, Huerta-Sánchez E, Sousa VC, Foll M (2013) Robust demographic inference from genomic and snp data. *PLOS Genetics*, **9**, 1–17.

Felsenstein J (1971) Inbreeding and variance effective numbers in populations with overlapping generations. *Genetics*, **68**, 581–597.

Franklin IR (1980) Evolutionary change in small populations. In *Conservation Biology: An Evolutionary Ecological Perspective* (edited by ME Soule, BA Wilcox), pp. 135–140. Sinauer Assocs, Sunderland, Mass.

Garcia-Cortes LA, Austerlitz F, de Cara MAR (2019) An evaluation of the methods to estimate effective population size from measures of linkage disequilibrium. *J Evol Biol*, **32**, 267–277.

Haldane J (1919) The combination of linkage values, and the calculation of distances between the lociof linked factors. *J Genetics*, **8**, 299–309.

Hayes BJ, Visscher PM, McPartlan HC, Goddard ME (2003) Novel multilocus measure of linkage disequilibrium to estimate past effective population size. *Genome Res.*, **13**, 635–643.

Hill W (1972) Effective size of populations with overlapping generations. *Theoretical Population Biology*, **3**, 278+.

Hill WG (1981) Estimation of effective population size from data on linkage disequilibrium. *Genet. Res.*, **38**, 209–216.

Hill WG, Robertson A (1968) Linkage disequilibrium in finite populations. *Theor and Appl Genet*, **38**, 226–231.

Lipshutz SE, Overcast IA, Hickerson MJ, Brumfield RT, Derryberry EP (2017) Behavioural response to song and genetic divergence intwo subspecies of white-crowned sparrows (*zonotrichia leucophrys*). *Molecular Ecology*, **26**, 3011–3027.

Loukas M, Krimbas C, Vergini Y (1979) Genetics of drosophila-subobscura populations .9. studies on linkage disequilibrium in 4 natural-populations. *Genetics*, **93**, 497–523.

Malpica J, Briscoe D (1982) Multilocus nonrandom associations in drosophila-melanogaster. *GENETICS RESEARCH*, **39**, 41–61.

Orive M (1993) Effective population size in organims with complex life-histories. *Theor. Pop. Biol.*, **44**, 316–340.

Piazza A, Mayr WR, Contu L, *et al* (1985) Genetic and population structure of four sardinian villages. *Annals of Human Genetics*, **49**, 47–63.

Robinson JD, Moyer GR (2013) Linkage disequilibrium and effective population size when generations overlap. *Evol Apps*, **6**, 290–302.

Santiago E, Novo I, Pardi<C3><B1>as AF, Saura M, Wang J, Caballero A (2020) Recent Demographic History Inferred by High-Resolution Analysis of Linkage Disequilibrium. *Molecular Biology and Evolution*, **37**, 3642–3653.

Sovic M, Fries A, Martin S, Lisle Gibbs H (2019) Genetic signatures of small effective population sizes and demographic declines in an endangered rattlesnake, *sistrurus catenatus*. *Evolutionary Applications*, **12**, 664–678.

Sved JA (1971) Linkage disequilibrium and homozygosity of chromosome segments in finite populations. *Theor Pop Biol*, **2**, 125–141.

Sved JA (2019) Linkage disequilibrium (ld). http://www.handsongenetics.com/PIFFLE/LinkageDisequilibrium.pdf.

Sved JA, Feldman MW (1973) Correlation and probability methods for one and two loci. *Theor Pop Biol*, **4**, 129–132.

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conservation Genetics*, **7**, 167–184.

Waples RS, Antao T, Luikart G (2014) Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics*, **197**, 769–780.

Waples RS, Do C (2008) LDNe: a program for estimating effective population size from data on linkage disequilibrium. *Mol Ecol Res*, **8**, 753–756.

Waples RS, Yokota M (2007) Temporal estimates of effective population size in species with overlapping generations. *Genetics*, **175**, 219–233.

Weir BS, Hill WG (1980) Effect of mating structure on variation in linkage disequilibrium. *Genetics*, **95**, 477–488.

## Appendix A: Note on relation between Sved (1971) and Sved & Feldman (1973)

Both methods are equivalent, but the first develops equations for $Q$ while the latter iterates $L$. As it seems $L = r^2$, both methods are equivalent if

$$L = \frac{1}{2N} + \left(1 - \frac{1}{2N}\right) Q. \tag{21}$$

Sved (1971) develops the following recursive equation

$$Q' = (1-c)^2 \frac{1}{2N} + (1-c)^2 \left(1 - \frac{1}{2N}\right) Q, \tag{22}$$

while Sved & Feldman (1973) develop recursive equations for

$$L^{'} = \frac{1}{2N} + (1-c)^2 \left(1 - \frac{1}{2N}\right) L. \tag{23}$$

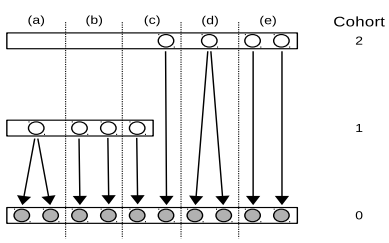
We can show this by replacing $L$ and $L^{'}$ in eq. (23) using eq. (21):

$$\frac{1}{2N} + \left(1 - \frac{1}{2N}\right) Q^{'} = \frac{1}{2N} + (1-c)^2 \left(1 - \frac{1}{2N}\right) \left[\frac{1}{2N} + \left(1 - \frac{1}{2N}\right) Q\right]$$

and, replacing $Q^{'}$, it coincides with eq. (22):

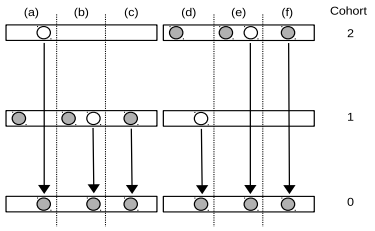
$$Q^{'} = (1-c)^2 \left[\frac{1}{2N} + \left(1 - \frac{1}{2N}\right) Q\right].$$

Figure 1: Inheritance of gametes in a scenario with two cohorts. Left: Case (a), two gametes (on gray) which are offspring of the same gamete one cohort removed. Case (b) two gametes are offspring from two independent gametes one cohort removed. Case (c) two gametes are offspring from gametes from two different cohorts. Case (d) two gametes are offspring from the same gamete two cohorts removed. Lastly, two gametes who descend from two different gametes two cohorts removed. Right: the probabilities of cases (a)-(e).




| | |
|---|---|
| (a) | $(1-c)^2\, p_1^2 \frac{1}{2N_1}$ |
| (b) | $(1-c)^2\, p_1^2 \left(1-\frac{1}{2N_1}\right) Q_{11}$ |
| (c) | $(1-c)^2\, 2p_1 p_2 Q_{12}$ |
| (d) | $(1-c)^2\, p_2^2 \frac{1}{2N_2}$ |
| (e) | $(1-c)^2\, p_2^2 \left(1-\frac{1}{2N_2}\right) Q_{22}$ |

Figure 2: Conditional IBD between both grey coloured gametes in an overlapping scenario (2 cohorts back). Cases (a-c) present the three components of the conditional IBD between two gametes of successive cohorts, cases (d-f) present the three components of the conditional IBD between two gametes two cohorts removed. The right hand side contains the probability of each case.




| | |
|---|---|
| (a) | $(1-c)\,p_2 Q_{12}$ |
| (b) | $(1-c)\,p_1\left(1-\frac{1}{2N_1}\right)Q_{11}$ |
| (c) | $(1-c)\,p_1\frac{1}{2N_1}$ |
| (d) | $(1-c)\,p_1 Q_{12}$ |
| (e) | $(1-c)\,p_2\left(1-\frac{1}{2N_2}\right)Q_{22}$ |
| (f) | $(1-c)\,p_2\frac{1}{2N_2}$ |

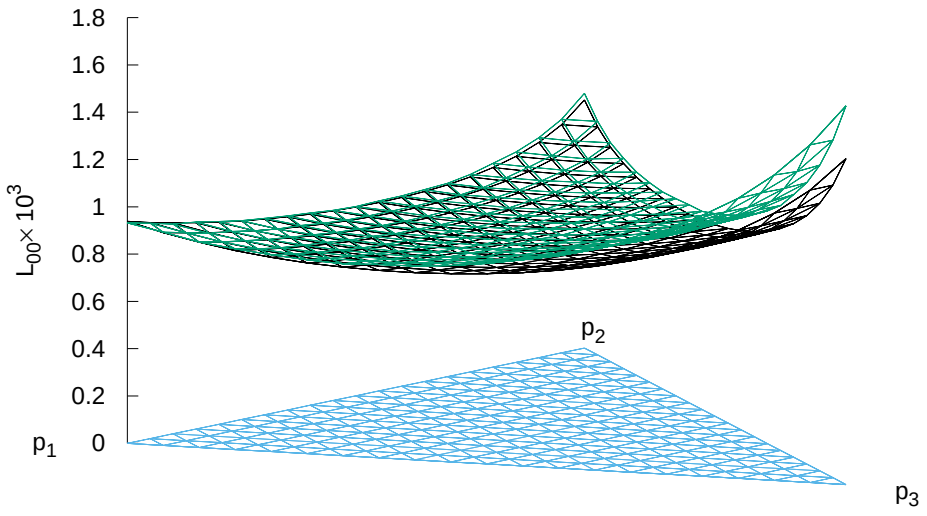



Figure 3: Equilibrium values for $L_{00}$ obtained via analytical results (black), simulations using multinomial distributions (green). $N_0 = 1000$ individuals and loci are assumed to be at 50cM. The triangle axis represent the probabilities of each newborn to come from each cohort $p_i$, which satisfy $\sum_{i=1}^{3} p_i = 1$.

Table 1: Results for $r^2$ evaluated for three scenarios, for different genetic distances ($d$) which correspond to different recombination rates ($c$). Results are given evaluated using multinomial distributions ("Empirical $r^2$"), via $Q_{00}$ or measured via $L_{00}$. Scenario 1: All contributions are the same (i.e. all $N_i$'s take the same value equal to 1000 diploid individuals) and $p_1 = p_2 = p_3 = \frac{1}{3}$. Scenario 2: All contributions are the same (all $N_i = 1000$ diploids), but $p_1 = 0.3$, $p_2 = 0.5$ and $p_3 = 0.2$. Scenario 3: All contributions are different: $N_1 = 1000$, $N_2 = 1600$, $N_3 = 500$ and $p_1 = 0.3$, $p_2 = 0.5$ and $p_3 = 0.2$.

| Scenario | $d$ (cM) | $c$ | Empirical $r^2 \times 10^3$ | $Q_{eq,00} \times 10^3$ | $L_{eq,00} \times 10^3$ |
|---|---|---|---|---|---|
| 1 | 5 | 0.0476 | 2.875 | 2.406 | 2.905 |
| 1 | 10 | 0.0906 | 1.657 | 1.166 | 1.666 |
| 1 | 20 | 0.1648 | 1.051 | 0.551 | 1.051 |
| 1 | 50 | 0.3161 | 0.701 | 0.201 | 0.701 |
| 2 | 5 | 0.0476 | 3.049 | 2.539 | 3.038 |
| 2 | 10 | 0.0906 | 1.733 | 1.234 | 1.733 |
| 2 | 20 | 0.1648 | 1.087 | 0.586 | 1.086 |
| 2 | 50 | 0.3161 | 0.713 | 0.216 | 0.715 |
| 3 | 5 | 0.0476 | 3.540 | 2.940 | 3.439 |
| 3 | 10 | 0.0906 | 1.994 | 1.435 | 1.935 |
| 3 | 20 | 0.1648 | 1.227 | 0.687 | 1.187 |
| 3 | 50 | 0.3161 | 0.772 | 0.257 | 0.757 |

Table 2: Results for estimated $N_0$ evaluated for three scenarios, for different genetic distances ($d$) which correspond to different recombination rates ($c$). Results are given evaluated using $r^2$ obtained via for $L_{00}$, using 100 and 500 pair of loci. Scenario 1: All contributions are the same (i.e. all $N_i$'s take the same value equal to 1000 diploid individuals) and $p_1 = p_2 = p_3 = \frac{1}{3}$. Scenario 2: All contributions are the same (all $N_i = 1000$ diploids), but $p_1 = 0.3$, $p_2 = 0.5$ and $p_3 = 0.2$. Scenario 3: All contributions are different: $N_1 = 1000$, $N_2 = 1600$, $N_3 = 500$ and $p_1 = 0.3$, $p_2 = 0.5$ and $p_3 = 0.2$.

| Scenario | $d$ | $c$ | Multi$\times 10^3$ | Mean | $SD_{100}$ | $SD_{500}$ |
|---|---|---|---|---|---|---|
| 1 | 5 | 0.0476 | 2.875 | 991.24 | 145.19 | 65.24 |
| 1 | 10 | 0.0906 | 1.657 | 996.31 | 143.85 | 63.40 |
| 1 | 20 | 0.1648 | 1.051 | 1005.66 | 145.45 | 63.97 |
| 1 | 50 | 0.3161 | 0.701 | 994.24 | 144.67 | 63.01 |
| 2 | 5 | 0.0476 | 3.049 | 996.67 | 145.50 | 62.01 |
| 2 | 10 | 0.0906 | 1.733 | 993.18 | 139.80 | 58.52 |
| 2 | 20 | 0.1648 | 1.087 | 999.98 | 145.68 | 63.53 |
| 2 | 50 | 0.3161 | 0.713 | 995.94 | 152.03 | 63.74 |
| 3 | 5 | 0.0476 | 3.540 | 956.48 | 141.35 | 60.35 |
| 3 | 10 | 0.0906 | 1.994 | 966.77 | 143.50 | 64.65 |
| 3 | 20 | 0.1648 | 1.227 | 968.07 | 138.63 | 60.84 |
| 3 | 50 | 0.3161 | 0.772 | 980.36 | 141.15 | 62.30 |

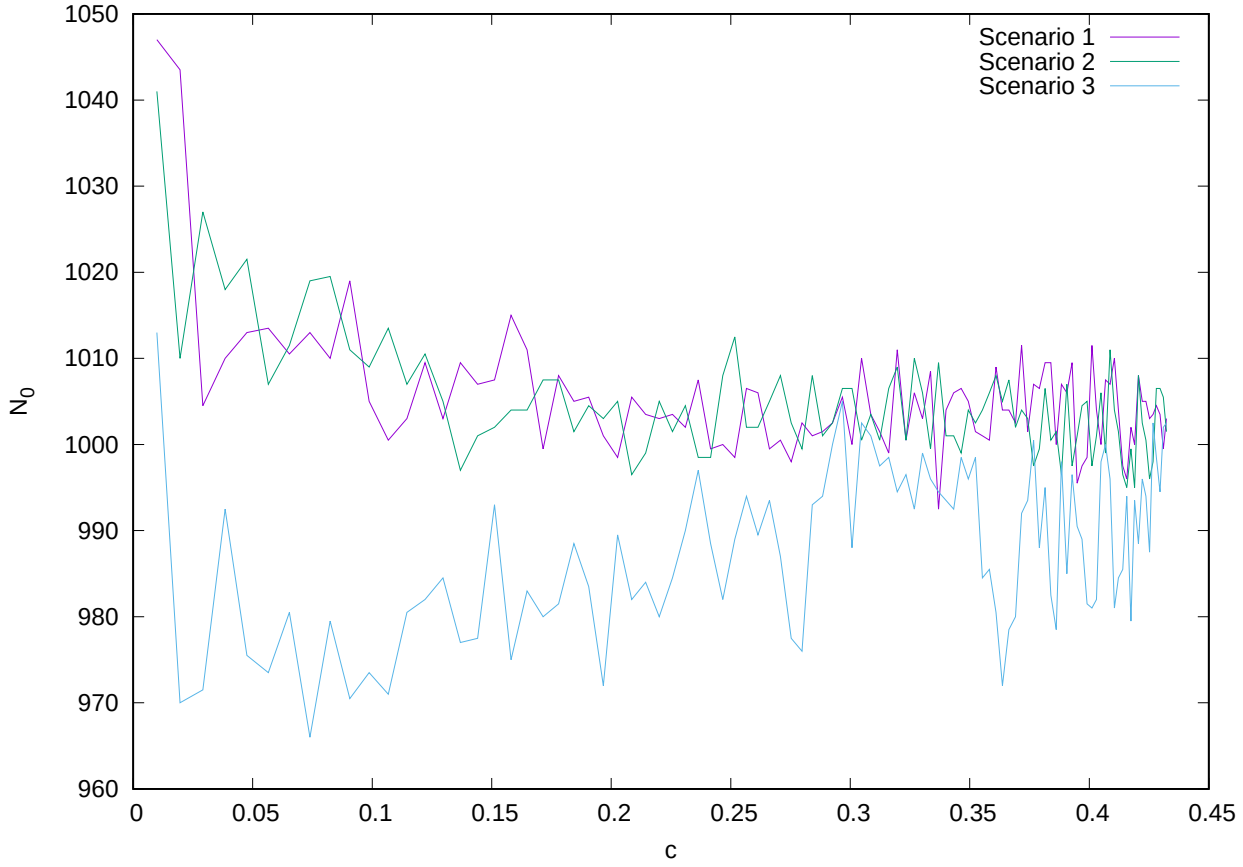



Figure 4: Inferred $N_0$ for a population with true $N_0 = 1000$ diploid individuals, under three different scenarios.

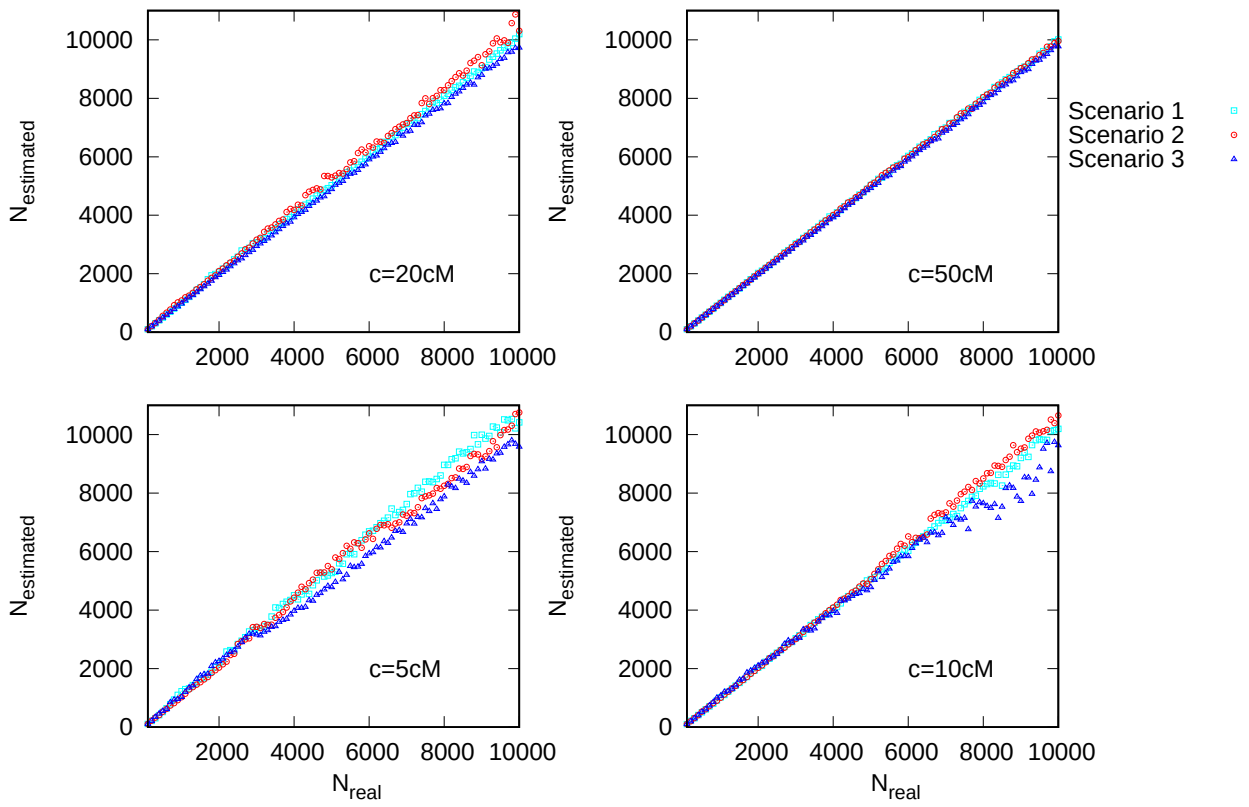



Figure 5: Inferred $N_0$ for a population with varying $N_0$ diploid individuals, under three different scenarios. Recombination distances are fixed at 5cM, 10 cM, 20 cM and 50 cM, from bottom to top.

| Individuals used | Harmonic mean $S$ | Corrected $R^2$ | $N_0$ | $N_e$ | `Ne estimator` |
|---|---|---|---|---|---|
| Nuttalli | 33 | 0.000766 | 1418.88 | 525.06 | 433.4 |
| Pugetensis | 71.1 | 0.000264 | 4117.43 | 1524.01 | 1260.6 |

Table 3: Estimated $N_0$ and $N_e$ for white-crowned sparrow, together with `Ne estimator` results (Waples & Do 2008).