Effective population size in *Drosophila subobscura*: ecological and molecular approaches

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The effective population size (Ne) represents the number of individuals that can contribute genes equally to the next generation and is usually smaller than the actual size of a population. The aims of this study were: (i) to assess the Ne for two *Drosophila subobscura* populations sampled from geographically close, but ecologically and topologically distinct habitats, (ii) to compare the results obtained from two independent approaches for estimating Ne [an ecological (capture-mark-release-recapture) and a molecular (microsatellite data, linkage disequilibrium and heterozygote excess)], and (iii) to obtain a long-term Ne estimation using a variety of mutational models for *D. subobscura* populations. The ecological method showed that the beech wood population (B) had a significantly larger Ne compared to the oak wood population (O). Observed sex ratio was in favor of females in both populations studied. The microsatellite analysis of populations showed that short-term effective population size in beech wood was larger when compared to population from oak wood, which is in concordance with results obtained by the ecological method. Long term Ne of both natural populations is infinite according to a variety of mutational models. Our results confirmed concordance between ecological and molecular methods in Ne estimation, but also suggested that ecological approach showed less robustness. Molecular approach provides a promising opportunity for more effective monitoring of Ne in *Drosophila subobscura*. However, the biology, demography and history of the populations may affect different estimators differently so we suggest that ecological and molecular approach should be combined in Ne estimation.

Key words: capture-mark-release-recapture, microhabitats, microsatellites, sex ratio.

INTRODUCTION

The effective population size (Ne) is a key concept in population genetics (Charlesworth, 2009). The rate of inbreeding and genetic drift is determined by Ne, which is defined as the number of individuals in an ideal population (i.e. with equal sex ratio and variance in family size approximated by a Poisson distribution) that would cause the same rate of inbreeding or genetic drift as that of the actual population in question (Frankham et al., 2002). The effective population size is usually far lower than the census numbers of breeding individuals in a species (Frankham, 1995). There is evidence that temporal fluctuations of Ne can occur in real populations, caused by and unequal sex ratio, high variance in family size and overlapping generations.

The Ne is an extremely useful concept for describing expected levels of genetic diversity and for evaluating the effect of different factors of the efficiency of selection (Petit & Barbadilla, 2009; Andolfatto et al., 2011). However it is important to note that it has
some limitations as a tool for understanding patterns of evolution and variation. For instance, certain aspects of genetic variability, such as frequency of individual nucleotide variants across different sites, cannot simply be described in terms of Ne (Charlesworth, 2009). Furthermore, reduction in variability caused by population bottleneck, a selective sweep or background selection might as well be associated with different variant frequency distributions and so can not be described by simple reduction in Ne (Braverman et al., 1995; Simonsen et al., 1995; Gordo et al., 2002; Kaiser & Charlesworth, 2009).

Monitoring the Ne, which is parallel to monitoring genetic variability, is of significant importance to population genetic studies and is applicable in conservation strategies. Field based research in combination with laboratory research can provide quick and valuable sources of data in such analyses (Frankham, 1999).

Direct counting of the exact number of individuals of organisms is difficult if not impossible. However, sampling of populations to estimate their size has become an extremely important component of studies in population dynamics and structure in ecology and evolution. Capture-mark-release-recapture technique is often used to estimate population size for species that are mobile (i.e. insects) and thus cannot be counted directly (Krebs, 1989). Ne can be safely estimated from the proportion of marked and unmarked individuals under the following assumptions (Southwood, 1978): (i) the marked animals are not affected (neither in behavior nor in life expectancy); (ii) the marked animals are completely mixed in the population; (iii) the probability of capturing a marked animal is the same as that of capturing any member of the population and (iv) sampling must be at discrete time intervals and the actual time involved in taking the samples must be small in relation to the total time.

In the genomic era advanced molecular techniques hold a great promise for the application of genetic markers to the estimation of Ne in different natural populations (Xu & Fu, 2004). The use of microsatellites, a class of highly polymorphic genetic markers is valuable in assessing genetic variation at the population level (e.g. Bruford & Wayne, 1993). Microsatellite loci are distributed throughout the genome and are generally neutral unless linked to loci under strong selection. The effective population size can be obtained from microsatellite data using short-term Ne estimation methods based on linkage disequilibrium (Hill, 1981) and heterozygote excess (Pudovkin et al., 1996). Also, assuming Wright-Fisher mutational model it is possible to obtain measures of expected heterozygosity (Ohta & Kimura, 1973), which together with mutation rate can successfully be applied to obtain estimation of the long-term Ne using different mutational models.

Drosophila subobscura represents an important model species in evolutionary biology research. It has a broad Palearctic distribution and also occurs on some Atlantic islands and in the late 1970s colonized South and North America where it spread rapidly and successfully to its new environments. As a consequence, this species has developed clines for some chromosomal arrangements and for wing size similar to those found in Old World populations (Prevosti et al., 1988; Huey et al., 2000; Gilchrist et al., 2001).

Numerous studies have focused on population genetic structure screening of different natural populations of D. subobscura, based on inversion polymorphism (Prevosti, 1974; Krimbas, 1993; Andjelković et al., 2007; Kenig et al., 2010), allozymes (Marinković et al., 1978; Pinto et al., 1997; Castro et al., 1999) and mtDNA (Afonso et al., 1990; Latorre et al., 1992; García-Martínez et al., 1998; Castro et al., 1999; Jelić et al., 2012).

Furthermore, D. subobscura displays rich microsatellite polymorphism in all acrocentric chromosomes of the set (Pascual et al., 2001). The characterization of these markers in Drosophila subobscura confirms their high variability (Pascual et al., 2000; Santos et al., 2010) and therefore makes them good candidates for a study of effective population size. Knowledge of Ne of D. subobscura populations originating from ecologically different habitats is important for understanding their current and future evolutionary potential for long term monitoring of microclimatic change in a habitat.

The aims of this study were: (i) to assess the effective population size for two D. subobscura populations sampled from geographically close, but ecologically and topologically distinct habitats, (ii) to compare Ne estimates obtained with an ecologically based (capture-mark-release-recapture) and a molecular based approach using microsatellite data (linkage disequilibrium and heterozygote excess), and (iii) to obtain a long-term Ne estimation of both population assuming that they represent a part of wider metapopulation of D. subobscura.
MATERIALS AND METHODS

Population samples

For the present study, *D. subobscura* flies were sampled from two localities (beech wood-B and oak wood-O) simultaneously at the end of June 2008. The beech wood-B (*Abieto fagetum*, 43°33′28.43′′N and 20°45′10.96′′E) and the oak wood-O (*Fraxinetto quercetum*, 43°32′57.38′′N and 20°40′02.32′′E) are both situated in Mountain Goč (Central Serbia). These two wood habitats are closely located (at different expositions, approximately 6 km far from each other), but have quite distinct microclimate conditions. Beech wood features higher humidity with dense vegetation coverage, whereas the Oak has sparser trees and is slightly warmer. These two *D. subobscura* populations (B and O) represent good model populations, since both display rich inversion polymorphism in all acrocentric chromosomes of the set. Some literature data confirmed that inversion polymorphism is, to a certain degree, associated with the ecological factors at the spatial and temporal level (Živanović et al., 1995; Andjelković et al., 2003; Savković et al., 2004; Stamenkovic-Radak et al., 2008). Furthermore, both populations showed significant fluctuation in effective population size across certain period of time (Stamenkovic-Radak et al., 2008).

Effective population size – ecological approach

Sampling was performed at both locations (B and O), in the same manner and with the same equipment. Flies were attracted by fermented fruit traps. Ten traps were set 10 m apart in a rectangular formation in the beech wood, and in a square formation in the oak wood in relation to the release point which is in the center of formation. The use of two different formations was necessary due to topography and steepness of terrain. The area covered by traps was thus 400 m² in each habitat and the total area of the study site was 7000 m². The total studied area was calculated according to Begon (1976) and Loukas & Krimbas (1979), where 30 m were added in each direction for each trap in order to take into account the range of dispersion and trap attraction of *D. subobscura* flies (Taylor et al., 1984). The potential effect of weather conditions at the site (e.g. temperature, humidity, wind) is acknowledged, because they may affect the assessment of Ne by the ecological method (Krimbas, 1993; Serra et al., 1987). The flies were captured with a net in the late afternoon peak of activity (between 18:00 and 20:30), counted without etherization, dusted with UV fluorescent dust (yellow) and released at a single release point, shortly before dusk. Late in the afternoon, on the day after release, flies were collected with the same sampling approach, brought to the lab and identified for the presence of fluorescent dust using UV light and a stereo microscope.

The species and sex of all recaptured flies were identified and flies were counted. The proportions obtained in recaptured samples were used to approximate numbers of flies of each species and sex released on the first day. Among the captured flies, *D. subobscura* was the dominant species, present at 85-95% in each sample. The species ratio and sex ratio were the same in two consecutive days.

The adapted and modified ecological method for estimating effective population size (Begon, 1977) was used. Population size for each sex (*Nm* and *Nf*) was determined (*N* = *rn/m*, where *r* is the total number of marked and released flies, *n* is the total number of recaptured flies, and *m* is the number of captured marked flies of each sex). Assuming that the flies have 100% survival between the time of release and the time of recapture (24 hrs), the effective population size was calculated using the following formula: *Ne* = (4*Nm* × *Nf*)/(*Nm* + *Nf*). *Ne* was estimated for one generation per year considering that our species is active from March to October.

Effective population size – molecular approach

For the microsatellite screening of populations, we used only the flies collected in the wild (38 and 51 individuals derived from B and O, respectively). The flies were kept frozen at –20°C prior to DNA extraction. In order to estimate the effective population size of the two *D. subobscura* populations, we conducted the fragment analysis for eleven microsatellite loci following Pascual et al. (2000). Each chromosome was represented with either two microsatellite loci (A: dsub05 and dsub19; U: dsub03 and dsub15; E: dsub13 and dsub20; J: dsub18 and dsub27), or three microsatellite loci for the longest chromosome (O: dsub01, dsub2 and dsub04) (Santos et al., 2010). Genomic DNA was extracted using the protocol of Martínez et al. (1992), slightly altered (the alkaline treatment was skipped). Prior to PCR reaction pureness and concentration of DNA isolates was determined on Eppendorf biophotometer. PCR was conducted in four multiplex reactions (dsub27, dsub20 and dsub04; dsub01, dsub18 and dsub05; dsub02, dsub13 and...
dsb19; and dsb03 and dsb15). Primer pairs used to amplify microsatellites were as in Pascual et al. (2000). Four different fluorescent dyes (FAM, NED, PET and VIC) were used to end-label one primer of each primer pair. PCR conditions were set following Pascual et al. (2001). A single soak at 95°C for 5 min was followed by 30 cycles of 1 min at 95°C, 30 s at 57°C and 30 s at 72°C. The exception was that a final elongation step of 30 min at 60°C was included and 2 µl (50 µg µl⁻¹) of DNA were added in the total volume of 20 µl of PCR mix. GeneScan500-LIZ size standard was used as an internal size marker. Fragment analysis was conducted on an ABI Prism 3130 automated sequencer. Only distinct and reproducible, well-marked amplified peaks were included in the genetic analysis. The obtained data were analyzed with the GeneMapper software (Applied Biosystems, Foster City, CA, USA).

Effective population size (Ne) was estimated from microsatellite data using short-term Ne estimation methods, namely linkage disequilibrium (Hill, 1981) and heterozygote excess (Pudovkin et al., 1996). The effective number of breeders using linkage disequilibrium data (Nbf(H)) was estimated using multiple pairs of unlinked loci. The standard linkage disequilibrium method (Hill, 1981) was recently shown to be biased when sample size is less than the true effective size (England et al., 2006). LDNE software (Waples & Do, 2008) contains an empirical correction that effectively eliminates the bias. The software can be downloaded from http://fish.washington.edu/xfer/LDNE/.

The effective number of breeders using Heterozygote Excess method (Nbf(H)) can be estimated examining the excess of heterozygotes in the sample compared to the proportion predicted under Hardy-Weinberg equilibrium (Pudovkin et al., 1996; Luikart & Cornuet, 1999). For the statistical data analysis we used NEESTIMATOR software (Peel et al., 2004; Ovenden et al., 2007, http://www.dpi.qld.gov.au/28_6908.htm).

The long-term Ne estimation (historical effective population size) on molecular data set was performed using expected heterozygosity assuming three different mutational models: (i) the infinite-alleles model [IAM: H = 4Neµ/(1 + 4Neµ); Kimura & Crow, 1964], (ii) the stepwise mutation model [SMM: H = 1 − (1 / (1 + 8Neµ))^(1/2); Ohta & Kimura, 1973] and, (iii) the single-step stepwise mutation model [SSMM: var(nA) = 4Neµ; Slatkin, 1995] was performed using data of variance of repeat number. The IAM assumes that each mutation creates a novel allele; the SMM assumes that new alleles arise by gain or loss of an integer number of repeat units; the SSMM consider that each mutation can increase or decrease the allele size by a single repeat, and it has been widely used as an approximation of the process underlying the genetic diversity at microsatellite loci (Goldstein et al., 1995; Slatkin, 1995; Zhivotovsky & Feldman, 1995).

The dinucleotide repeat mutation rate of Drosophila melanogaster (9.3 × 10⁻⁶ per locus per generation) empirically determined by Schug et al. (1998) was used, since it produces estimates of Ne concordant with those obtained from sequences of single nuclear genes (Pascual et al., 2000). By rearranging the equations, solving for Ne at each locus, and averaging across all 11 polymorphic loci, we obtained estimates for both sampled populations of D. subobscura. The expected heterozygosity for each of 11 loci for both populations was calculated in Arlequin 3.5. Software (http://cmpg.unibe.ch/software/arlequin35/Arl35Downloads.html, Excoffier et al., 2005). The variance in repeat number for each of loci was calculated using the freely download MICROSAT Software (http://genetics.stanford.edu/hpgl/projects/microsat/). The values of both the parameters (H and var(nA)) were evaluated for significance using non-parametric Mann-Whitney U test with the free download software PAST (http://folk.uio.no/ohammer/past/, Hammer et al., 2001).

**RESULTS**

The results of the ecological approach to estimate population size (N), effective population size (Ne) and their ratio (Ne/N) in two populations for both sexes are presented in Table 1. The results show that females outnumbered males in both B and O populations. The effective population size in beech popula-

<table>
<thead>
<tr>
<th></th>
<th>Nf</th>
<th>Nm</th>
<th>N</th>
<th>Ne</th>
<th>Ne/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beech (B)</td>
<td>3419.18</td>
<td>1976.8</td>
<td>5395.98</td>
<td>5010.42</td>
<td>0.93</td>
</tr>
<tr>
<td>Oak (O)</td>
<td>106.55</td>
<td>35.87</td>
<td>142.42</td>
<td>107.34</td>
<td>0.75</td>
</tr>
</tbody>
</table>

**TABLE 1. Ecological approach: Population size (N), effective population size (Ne) and their ratio in B (beech) and O (oak) population of D. subobscura. Nf: number of females; Nm: number of males**
TABLE 2. Details and variability of the microsatellite loci in Beech (B) and Oak (O) *D. subobscura* populations

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allelic size range (bp)</th>
<th>Chr.</th>
<th>Alleles</th>
<th>HO_E</th>
<th>p (χ² test)</th>
<th>Alleles</th>
<th>HO_E</th>
<th>p (χ² test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsub05</td>
<td>130-183</td>
<td>A</td>
<td>15</td>
<td>0.8571</td>
<td>0.9245</td>
<td>0.0049</td>
<td>13</td>
<td>0.7586</td>
</tr>
<tr>
<td>dsub04</td>
<td>176-214</td>
<td>O</td>
<td>17</td>
<td>0.7895</td>
<td>0.9014</td>
<td>0.0139</td>
<td>16</td>
<td>0.7708</td>
</tr>
<tr>
<td>dsub18</td>
<td>193-231</td>
<td>J</td>
<td>17</td>
<td>0.8684</td>
<td>0.8775</td>
<td>0.0001</td>
<td>13</td>
<td>0.7872</td>
</tr>
<tr>
<td>dsub27</td>
<td>213-266</td>
<td>J</td>
<td>20</td>
<td>0.8947</td>
<td>0.9126</td>
<td>0.0004</td>
<td>17</td>
<td>0.6735</td>
</tr>
<tr>
<td>dsub20</td>
<td>237-295</td>
<td>E</td>
<td>17</td>
<td>0.5790</td>
<td>0.8407</td>
<td>0.0815</td>
<td>15</td>
<td>0.3256</td>
</tr>
<tr>
<td>dsub01</td>
<td>257-289</td>
<td>O</td>
<td>15</td>
<td>0.7632</td>
<td>0.8811</td>
<td>0.0158</td>
<td>13</td>
<td>0.8913</td>
</tr>
<tr>
<td>dsub03</td>
<td>131-162</td>
<td>U</td>
<td>13</td>
<td>0.8684</td>
<td>0.8211</td>
<td>0.0027</td>
<td>11</td>
<td>0.9546</td>
</tr>
<tr>
<td>dsub13</td>
<td>99-150</td>
<td>E</td>
<td>15</td>
<td>0.9474</td>
<td>0.8351</td>
<td>0.0151</td>
<td>13</td>
<td>0.6222</td>
</tr>
<tr>
<td>dsub19</td>
<td>174-218</td>
<td>A</td>
<td>11</td>
<td>0.7143</td>
<td>0.8873</td>
<td>0.0337</td>
<td>9</td>
<td>0.7500</td>
</tr>
<tr>
<td>dsub02</td>
<td>203-265</td>
<td>O</td>
<td>18</td>
<td>0.7632</td>
<td>0.9197</td>
<td>0.0266</td>
<td>12</td>
<td>0.8837</td>
</tr>
<tr>
<td>dsub15</td>
<td>225-269</td>
<td>U</td>
<td>16</td>
<td>0.7632</td>
<td>0.8891</td>
<td>0.0178</td>
<td>12</td>
<td>0.8182</td>
</tr>
</tbody>
</table>

Σ = 8.8082, Σ = 9.6900, Σ = 0.2125, p = n.s.

HO: observed heterozygosity, HE: expected heterozygosity, p: probability level for Hardy-Weinberg equilibrium (df = 10)

TABLE 3. Effective number of breeders in *D. subobscura* populations estimated with disequilibrium (*Nb* (D)) and heterozygote excess (*Nb* (H)) methods. S: sample size; L: number of loci; \( \tau^2 \): mean squared frequencies of all pairs of loci; 95% CI: confidence intervals for Ne estimates

<table>
<thead>
<tr>
<th>Population</th>
<th>S</th>
<th>L</th>
<th>( \tau^2 )</th>
<th><em>Nb</em> (D)</th>
<th>95% CI</th>
<th><em>Nb</em> (H)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beech (B)</td>
<td>38</td>
<td>11</td>
<td>0.03617</td>
<td>154</td>
<td>110.3-245.8</td>
<td>infinite</td>
<td>not available</td>
</tr>
<tr>
<td>Oak (O)</td>
<td>51</td>
<td>11</td>
<td>0.03138</td>
<td>77</td>
<td>62.7-97.8</td>
<td>21.1</td>
<td>not available</td>
</tr>
</tbody>
</table>
tion is higher (46x) compared to Ne in oak. The ratio Ne/N for B population showed high value (0.93) which suggests that effective population size is close to census size. However, in O population the ratio Ne/N showed lower value then 0.8 which might have been caused by a strongly unbalanced sex ratio in this particular year.

The microsatellite variability data represented by expected heterozygosity and variance in repeat number for locus/loci are presented in Table 2. The mean expected heterozygosity showed high values in both populations (0.830 and 0.881 for O and B, respectively). Population of D. subobscura beech wood had greater expected H value than oak population from the same mountain (Mann-Whitney U test: 0.881 vs 0.830, Z = –2.101, p = 0.034). The beech population showed higher variance in repeat number then oak populations (Mann-Whitney U test: 7.77 vs 3.72, Z = –2.896, p = 0.002).

The estimation of short-term effective number of breeders using linkage disequilibrium (LD) and heterozygote excess methods on microsatellite data are shown in Table 3. The results of LD analysis showed that Beech population size [Nb (D) = 154, CI (95%) = 110.3 – 245.8] is twice higher compared to size of Oak population [Nb (D) = 77, CI (95%) = 62.7 – 97.8]. The heterozygote excess method showed that Beech population has infinite effective number of breeders, however Oak population showed smaller effective population size (Nb (H) = 21).

The estimation of long-term (historical) effective population size using microsatellite data due to three mutational models [infinite alleles model (IAM), step-wise mutation model (SMM) and single-step stepwise mutation model (SSMM)] are presented in Table 4.

The results based on microsatellite analysis showed the higher effective population size in Beech population compared to Oak but with different ratios for three mutation models (1.44x, 1.87x and 2.16x for IAM, SMM and SSMM, respectively). Also, the results showed extremely high values of effective population size for both sampled populations of D. subobscura.

**DISCUSSION**

The aim of the present study was to assess the effective population size (Ne) using two independent approaches: ecological and molecular. Ne represents the average size of a population in terms of the number of individuals that can contribute genes equally to the next generation and is usually smaller than the actual population size. Theoretically, the ecologically based approach could be useful to understand the effect of demographic characteristics of population (population size, sex ratio, mortality, etc.) and to assess the changes in effective population size caused by microhabitat and/or microclimatic variation, while the molecular approach provides an estimation of the expected rate of loss of genetic variation, the rate of increase in inbreeding, and the strength of selection required to counter the effect of genetic drift (Crow & Denniston, 1988).

The presented results of effective population size estimated by ecological method (capture-mark-release-recapture) showed that beech (B) population...
has a significantly larger effective population size compared to oak (O) population which fit into data obtained for several years for the two populations under study (Stamenkovic-Radak et al., 2008). The effective population size of *D. subobscura* in beech wood can be considered as infinite (more that 4000 breeders) according to Wright (1943) and Begon *et al.* (1980). However, the population sampled in oak wood showed extremely low effective population size (less than 1000 individuals) and this was the lowest effective population size measured at that locality in the last eleven years (Stamenkovic-Radak *et al.*, 2008). Extremely unequal sex-ratio and fluctuating population census, detected in the oak wood population, could lead to reduction in effective population size and consequently to reduction of genetic diversity and reproductive fitness of population (Woodworth *et al.*, 1994).

The differences between numbers of each sex of *D. subobscura* obtained in this study are large in both populations, and have an obviously significant effect on *Ne*. The sex ratio differs between populations and is more unbalanced towards females in oak wood population. The abundance of females over males in both populations was detected also in previous seasons at both localities (Stamenkovic-Radak *et al.*, 2008). That could be the result of the fact that females showed higher longevity than males (Christie *et al.*, 2004). However, Christie *et al.* (2004) presented results obtained in controlled laboratory conditions and such explanation cannot be fully applied to natural conditions. Furthermore, the occurrence of biased sex-ratio can be of considerable ecological and evolutionary importance. This bias can result from X-linked meiotic drive that affects Y-bearing sperm and causes males to produce female progeny. This trait was described in several *Drosophila* species (Jaenike, 2001). Contrary to our findings, Pascual *et al.* (2004) presented that wild samples of *D. subobscura* yielded a significantly higher number of males than females during those months, when the species is more abundant. In other cases, they detected no significant deviation of 1:1 in sex proportion in wild populations. The short-term effective population size obtained using microsatellite data of both sampled *D. subobscura* population is in concordance with previous literature data for lethal allelism for *D. subobscura* (Begon *et al.*, 1980; Loukas *et al.*, 1980; Mestres *et al.*, 1990; Zivanovic *et al.*, 2007; Araúz *et al.*, 2009). Most of these authors (Begon *et al.*, 1980; Loukas *et al.*, 1980; Mestres *et al.*, 1990; Araúz *et al.*, 2009) suggested that the population of *D. subobscura* is extremely large and effectively infinite. However, Zivanovic *et al.* (2007) found that the *Ne* values varied greatly from 370 to 19413 and that was attributed to the habitat conditions and some environmental factors. In our study, both short-term methods for *Ne* estimation (Linkage Disequilibrium and Heterozygote Excess methods) performed on microsatellite data showed

**TABLE 5. Comparison of ecological and molecular approaches**

<table>
<thead>
<tr>
<th></th>
<th>Ecological approach</th>
<th>Molecular approach</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description:</strong></td>
<td>Usefull to asseses the demographic <em>Ne</em> and to understand the effect of demographic characteristics of population (population size, sex ratio, mortality etc.) and to detect the changes in effective population size caused by microhabitat and/or microclimatic variation</td>
<td>Provides an estimation of the expected rate of loss of genetic variation, the rate of increase in inbreeding, and the strength of selection required to counter the effect of genetic drift by using short and long term evaluation of <em>Ne</em></td>
</tr>
<tr>
<td><strong>Field/laboratory work:</strong></td>
<td>field work field and laboratory work</td>
<td></td>
</tr>
<tr>
<td><strong>Output information:</strong></td>
<td><em>N, Ne, N/Ne, sex ratio, demographic parameters</em></td>
<td><em>Ne, migration rate, bottleneck evaluation</em></td>
</tr>
<tr>
<td><strong>Sample size:</strong></td>
<td>Large sample size</td>
<td>Small/moderate sample size</td>
</tr>
<tr>
<td><strong>Cost:</strong></td>
<td>cheap</td>
<td>expensive</td>
</tr>
<tr>
<td><strong>time consuming</strong></td>
<td></td>
<td>efficient</td>
</tr>
<tr>
<td><strong>Disadvantages:</strong></td>
<td>Fluctuation in weather condition (temperature, precipitation, wind etc.) during sampling and re-sampling</td>
<td><em>Ne</em> valuation dependence of short or long term evaluation of effective population size</td>
</tr>
</tbody>
</table>
significantly larger population size in beech wood compared to population from oak wood which is also in concordance with results obtained by ecological method. However, it should be noted that different methods are not necessarily estimating the same Ne because they are subjected to different bias. The heterozygosity excess methods estimate the effective size of the parental population; the LD methods infer the short- to intermediate-term (mean) effective population size, the length of the term being dependent on the linkage between markers, while the methods considering the mutational process explicitly estimate the long-term Ne in the past on a time-scale of the order of Ne generations.

The long term Ne estimates from current genetic variation of beech and oak populations have been derived independently from a variety of mutational models (IAM, SMM and SSMM) and we have detected the same Ne ratio between populations but three different Ne values. Colson & Goldstein (1999) suggested that most alleles are generated by gain or loss of a repeat unit according to SMM model, while some alleles originate by more complex mutations, as observed by Van Oppen et al. (2000) using a different approach. For microsatellites, the widely used mutation model is the single stepwise mutation model (SSMM; Ohta & Kimura, 1973), which assumes that a mutation leads to one repeat unit increase or decrease in allele size with an equal probability. The effective population size of both populations in the present study evaluated using three models predicted significantly larger Ne in beech compared to oak population. Therefore, based on microsatellite data, it is quite clear that the long-term Ne of these natural populations of D. subobscura is extremely large.

Regarding molecular approach, it is important to understand the time-scales behind each method, because natural populations rarely have constant Ne; rather, they are dynamic entities changing significantly in size and distribution over time. Therefore, using the same data, different methods could yield considerably different estimates of Ne. Additionally, the biology, demography and history of the populations may affect different estimators differently. Ne is a parameter summarizing the effects of many other demographic parameters in determining a given genetic property of the population (Caballero, 1994). In some cases, such a highly summary parameter is desirable, simplifying both the explanation of the pattern and amount of genetic variation observed in a population and the prediction of the genetic properties (such as loss of variation, fixation probability, changes in fitness of the population) in the future. In other cases, however, it is more helpful to know the details that determine Ne. This is especially true in conservation biology, where appropriate management can be exercised only when detailed knowledge of the population is available. In the present scheme, we showed the advantage/disadvantage of both, ecological and molecular approach (Table 5).

Finally, our results suggest that both, ecological and molecular approaches combined provide a great opportunity for more precise monitoring of effective population size in Drosophila subobscura. However, in general, careful consideration of the natural history of each particular population or species must also dictate the selection of the most appropriate analytical method for Ne monitoring efforts.

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