Genetically effective and adult population sizes in the Apache silverspot butterfly, *Speyeria nokomis apacheana* (Lepidoptera: Nymphalidae)

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GENETICALLY EFFECTIVE AND ADULT POPULATION SIZES IN THE APACHE SILVERSPOt BUTTERFLY, SPEYERIA OKOMIS APACHEANA (LEPIDOPTERA: NYMPHALIDAE)

Hugh B. Britten1, Erica Fleishman2, George T. Austin3, and Dennis D. Murphy4

ABSTRACT.—Genetically effective and adult population sizes of the Apache silverspot butterfly (Speyeria nokomis apacheana) from the central Great Basin of North America were tracked from 1991 to 1997. Speyeria nokomis inhabits mesic habitats in the Great Basin, and its recent declines in abundance have raised concerns about its future persistence. Adult population size estimates determined from mark-recapture methods ranged from 100 to 1900 individuals from 1993 through 1997. Genetically effective population sizes based on temporal changes in allozyme frequencies ranged from less than 1 individual to 20 individuals between 1991 and 1995. Despite these small annual estimates, it is likely that gene flow resulted in overestimation of genetically effective population sizes. These results, combined with observations in the field, suggest that suitable but vacant habitat patches be maintained for potential recolonization by S. n. apacheana.

Key words: gene flow, metapopulation, allozymes.

Much recent research in conservation biology has focused on the effects of population size fluctuations on genetically effective population sizes (Ne; Woodworth et al. 1994, Frankham 1995, 1996, Vucetich et al. 1997, Kali­nowski and Waples 2002). It is now generally recognized that fluctuations in population size are the main cause of reduction of Ne, which is usually expressed as the ratio of effective to census population sizes (Ne/Ne; Frankham 1996, Vucetich et al. 1997). A reduction in Ne/Ne results in the erosion of population genetic variability within just a few generations (Frankel and Soule 1981, Allendorf 1986, Hedrick and Miller 1992; genetic variability in populations is usually expressed as mean individual heterozygosity and/or allelic diversity). Because population size fluctuation is a crucial determinant of Ne, and because sizes of natural insect populations are known for their extreme fluctuations (Ehrlich and Murphy 1987), long-term genetic and demographic studies of insect populations can be especially useful in understanding the dynamics of Ne/Ne and the effect of those dynamics on genetic variability within populations. Although demographic and environmental factors have profound effects on species persistence in the short term (Lande 1988), genetic diversity generally is believed to enable species to persist over evolutionary time (Allendorf 1986).

Population dynamics and life histories of butterflies differ fundamentally from those of vertebrates. Long generation times may allow some vertebrate populations to retain genetic variability for considerable periods during declines in abundance (Dinerstein and McCracken 1990), but most butterfly populations have relatively short generation times which, when combined with fluctuating population sizes, expose them to potentially rapid loss of variability due to genetic drift. Under such circumstances, dispersal between occupied habitat patches can be vital to the persistence of these populations (Ehrlich and Murphy 1987, Britten et al. 1994a). Although demographic and genetic parameters may be more difficult to estimate in insects than in vertebrates, understanding the relationships between census population size, genetically effective population size, and rates of dispersal can be critical in managing rare and imperiled invertebrates, including butterflies.

Herein we report results of a study in which both census and effective population sizes were tracked over several years in a population of a
butterfly of conservation concern from the central Great Basin. We also discuss management implications of this work.

**Biology of Speyeria nokomis apacheana**

Our work focused on *Speyeria nokomis apacheana* (Skinner) (Lepidoptera: Nymphalidae; Apache silverspot butterfly), a taxon generally confined to small habitat patches, including insular seeps, springs, and riparian areas across the central and western Great Basin (Austin 1985). Research was prompted both by concerns that the subspecies might warrant federal protection and by its suitability as a model for studying how isolation and population fluctuations affect genetic variability in natural populations. Concerns for the persistence of *S. n. apacheana* have been heightened by the recent loss of at least 2 western Nevada populations of *S. n. carsonensis*, a candidate for protection under the U.S. Endangered Species Act. *Speyeria nokomis* is ideal for estimating Ne and examining how fluctuations in its abundance affect genetic diversity because the species is univoltine with nonoverlapping generations (Scott and Mattoon 1981, Scott 1986), appears to have a 1:1 sex ratio, and has levels of gene flow between populations that are low, but detectable (Britten et al. 1994b). Genetic and mark-recapture (MRR) data suggest that *S. n. apacheana* exists as a metapopulation (a set of local populations linked by dispersal) in parts of the Toiyabe Range (Britten et al. 1994b, Fleishman et al. 2002). We collected butterflies for allozyme analysis during 1991–1996 and made mark-recapture–based population size estimates over 5 years (1993–1997). Together, these data allow estimation of Ne/N based on both demographic and genetic data, where N is the estimated annual adult population size. Goals of the study were to provide relatively long-term data on the dynamics of Ne/N, heterozygosity, and allelic diversity for *S. n. apacheana*.

**Methods**

We performed mark-recapture sampling for 3 to 10 days each flight season of the study (Table 1). Sampling effort was spread throughout each flight season to incorporate as well as possible within-season fluctuations in adult population size. Mark-recapture data were collected from 1993 to 1997 from a population of *Speyeria nokomis apacheana* near the Reese River in Lander County, Nevada (see Britten et al. 1994b for exact location). Sampling techniques followed those developed by Ehrlich and Davidson (1960). Adult population size estimates (N) were calculated with Jolley 3.6 (Dunn 1997, hellmann@zoology.ubc.edu to obtain a copy of this software), a Microsoft Excel® program based on the algorithm of Jolly (1965). The Jolly algorithm assumes constant survival and recapture probabilities. That is, individuals that have been captured previously, and those that have not, are equally likely to be captured on a given sample day. Jolley 3.6 uses a linear interpolation method of daily population size estimates to extrapolate annual population sizes for adults using Scott's (1973) method I for each year of sampling. Although sex ratio in *S. nokomis* is believed to be 1:1, females fly later and exhibit more cryptic behavior than males; therefore, recapture data were treated separately for males and females (Tabachnik 1980, Wilcox et al. 1986). Estimates were calculated for males and then doubled for the entire population.

We collected adult butterflies in late August and early September each year by netting. Specimens were immediately frozen in liquid nitrogen and then transported to the laboratory. Standard methods of specimen storage, preparation, and electrophoretic analysis were used as described for this butterfly (Britten et al. 1994b). BIOSYS-1 (Swofford and Selander 1981) was used to estimate levels of heterozygosity for each year's sample. Ne's for the years 1991–1995 were estimated using Pollak's (1983) and Waples' (1989) methods based on allele frequency changes from one generation to the next. Ne's were estimated in a pairwise sequential fashion. Sample plan I, where sampling occurs after reproduction (Waples 1989), was...
TABLE 1. Results of mark-recapture efforts by year for Reese River, central Great Basin, Speyeria nokomis apacheana. M indicates number of males captured, F indicates females captured, MR indicates number of recaptured males, and FR is recaptured females.

<table>
<thead>
<tr>
<th>Year</th>
<th>M</th>
<th>MR (%)</th>
<th>F</th>
<th>FR (%)</th>
<th>Total</th>
<th>Total recapture (%)</th>
<th>Days sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>41</td>
<td>37 (90)</td>
<td>11</td>
<td>6 (55)</td>
<td>52</td>
<td>43 (83)</td>
<td>8</td>
</tr>
<tr>
<td>1994</td>
<td>157</td>
<td>106 (68)</td>
<td>31</td>
<td>18 (58)</td>
<td>188</td>
<td>124 (66)</td>
<td>5</td>
</tr>
<tr>
<td>1995</td>
<td>600</td>
<td>419 (70)</td>
<td>207</td>
<td>74 (35)</td>
<td>807</td>
<td>493 (61)</td>
<td>10</td>
</tr>
<tr>
<td>1996</td>
<td>70</td>
<td>55 (79)</td>
<td>20</td>
<td>13 (65)</td>
<td>90</td>
<td>68 (76)</td>
<td>6</td>
</tr>
<tr>
<td>1997</td>
<td>21</td>
<td>6 (29)</td>
<td>9</td>
<td>2 (22)</td>
<td>30</td>
<td>8 (27)</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>889</td>
<td>623 (70)</td>
<td>278</td>
<td>113 (41)</td>
<td>1167</td>
<td>736 (63)</td>
<td></td>
</tr>
</tbody>
</table>

assumed based on behavioral observations (Scott 1986).

RESULTS

The number of males captured each year ranged from 21 in 1997 to 600 in 1995, and the number of females from 11 in 1993 to 74 in 1995 (Table 1). Total recapture rate for the duration of the study was 63%, and annual recapture rates ranged from 22% for females in 1997 to 90% for males in 1993 (Table 1). Annual size of the Reese River Speyeria nokomis apacheana population fluctuated by more than one order of magnitude (100–1900) from 1993 to 1997 based on MRR data (Table 2). Effective population sizes based on allele frequency data for 5 polymorphic loci (Table 3) were <10 each generation from 1991 through 1995 based on the Pollak (1983) method, and ≤20 each generation based on Waples’ (1989) method (Table 2). The 95% confidence intervals for both types of estimates overlapped broadly and were considerably larger than individual estimates (Table 2). Ratios of Ne to adult population size (N) for 1993–1995 ranged from 0.001 to 0.200 (Table 2). The long-term Ne/N estimate for the period 1993 through 1997 based on fluctuations in adult population sizes (Vucetich et al. 1997) was 0.382.

Allelic diversity (Table 3) in the study population was substantially more affected by population size fluctuations than was heterozygosity (Table 2). Three alleles, GPI-1d, MDH-1b, and PGD-1d, were detected at low frequencies in 1992 within heterozygous individuals (Table 3). These alleles were not detected in 1991 although our large sample size of 58 butterflies provided probabilities of 0.86, 0.97, and 0.97 for detecting GPI-1d, MDH-1b, and PGD-1d, respectively, at the frequencies observed in 1992. Thus, we are confident that our 1991 sample size was adequate to detect these alleles had they been segregating in the population that year. Hardy-Weinberg mean individual heterozygosity ranged from 0.021 to 0.054, and observed mean heterozygosity ranged from 0.017 to 0.061 (Table 2).

DISCUSSION

Census population fluctuations such as those observed in Speyeria nokomis apacheana can be viewed as genetic bottleneck events through which many populations must inevitably pass. Fluctuations in size increase the probability that population heterozygosity will decrease over time and that alleles will be lost by genetic drift. Allendorf (1986) pointed out that short-term bottlenecks may have little effect on heterozygosity, yet can profoundly affect allelic diversity. Because heterozygosity can be preserved when a population passes through a narrow bottleneck of short duration, the population's short-term persistence may not be significantly compromised by the inbreeding-like effects of drift during bottlenecks. The parameter used to estimate the impact of population bottlenecks on genetic variability, Ne, is a theoretical construct that is subject to several assumptions that are difficult to meet in natural populations (see Waples 1989, Nunney 1995). Thus, few estimates of Ne are available from natural populations (see Frankham 1995). This long-term study provided us an opportunity to track adult and genetically effective population sizes for S. n. apacheana and to observe effects of genetic drift and gene flow on heterozygosity and allelic diversity in a population of this butterfly.

The assumption of a closed population necessary for estimating Ne is clearly violated in this butterfly and in similar studies (e.g., olive...
TABLE 2. Estimated adult population sizes (N), variance in effective population sizes calculated after Pollak (1983; $N_e$P) and Waples (1989; $N_e$W), Hardy-Weinberg expected heterozygosity ($H_e$), and observed heterozygosity ($H_o$) in *Speyeria nokomis apacheana* from the Reese River, central Great Basin.

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>$N_e$P</th>
<th>$N_e$W</th>
<th>$N_e$/N</th>
<th>$N_e$/W</th>
<th>$H_e$</th>
<th>$H_o$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>100</td>
<td>(0-11)</td>
<td>(1-23)</td>
<td>0.010</td>
<td>0.020</td>
<td>0.037</td>
<td>0.039</td>
</tr>
<tr>
<td>1992</td>
<td>300</td>
<td>(2-24)</td>
<td>(1-13)</td>
<td>0.030</td>
<td>0.001</td>
<td>0.041</td>
<td>0.035</td>
</tr>
<tr>
<td>1993</td>
<td>1,900</td>
<td>&lt;1</td>
<td>(18-53)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.043</td>
<td>0.043</td>
</tr>
<tr>
<td>1994</td>
<td>1,000</td>
<td>(2-26)</td>
<td>(1-68)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.061</td>
<td>0.061</td>
</tr>
<tr>
<td>1995</td>
<td>200</td>
<td>7</td>
<td>(0-11)</td>
<td>0.024</td>
<td>0.001</td>
<td>0.054</td>
<td>0.054</td>
</tr>
<tr>
<td>1996</td>
<td>200</td>
<td>2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.021</td>
<td>0.021</td>
</tr>
<tr>
<td>1997</td>
<td>209</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.017</td>
<td>0.017</td>
</tr>
</tbody>
</table>

flies; Krimbas and Tsakas 1971). Local sampling of a widely distributed population may result in inaccurate estimates of allele frequencies for the entire population. If population substructure is present, the likely result will be underestimation of $N_e$ for the entire population (Nei and Tajima 1981, Pollak 1983). Conservation biologists, however, are often interested in determining $N_e$ for a particular isolated population or set of populations within a metapopulation. This is the case for the Reese River population of *S. nokomis*. Here it is crucial that population structure be well understood because migration from outside the population of interest will inflate $N_e$ estimates. Because of these considerations, we can be certain that $N_e$ was overestimated in the Reese River *S. n. apacheana* population during at least some years of this study. The loss of rare alleles with high probabilities of detection in one generation and subsequent reappearance in another strongly suggests that dispersal into the Reese River population occurred, at least sporadically, during the study (Table 3). Furthermore, Britten et al. (1994b) estimated that *S. n. apacheana* populations exchanged an average of 0.85 genetically effective migrants per generation among populations sampled across the western Great Basin in 1991. Most likely, levels of gene flow vary from year to year, and this results in overestimation of $N_e$ some years and more accurate estimates for other years.

Nearly 40 patches of suitable habitat for *S. n. apacheana* have been identified in the nearby Toiyabe Range, several of which are fewer than 20 km from the Reese River site (Fleishman et al. 2002). Considerable variation in patch occupancy has been documented in the Toiyabe Range over the past 5 years, with annual turnover rates ranging from 13% to 49% (Fleishman et al. 2002). Although a few patches may support self-sustaining populations of *S. n. apacheana*, census population sizes in some patches were below 10 individuals in each year of occupancy. This observation strongly suggests that migration plays an important role in the long-term persistence of the butterfly. These results, and those of previous work (Britten et al. 1994b), suggest an overall management strategy for *S. n. apacheana* in the central Great Basin. It is clear that individual populations will fluctuate widely in abundance, increasing their vulnerability to potential extirpation driven by demographic and environmental stochasticity. It is also apparent that populations on isolated habitat patches may be genetically and demographically augmented by immigrants. The potential for this "rescue effect" (Brown and Kodric-Brown 1977) makes it imperative that habitat for the butterfly be managed in a way that recognizes interconnections between patches. Habitat patches that have lost their *S. n. apacheana* populations should be suitably maintained as butterfly habitat for a number of years to facilitate possible recolonization from occupied patches. Sites that maintain large populations, such as the Reese River site, should be documented, monitored, and maintained as potential source populations for nearby patches.

The Reese River *S. n. apacheana* population exhibits several attributes typical of butterfly populations that are confined to insular habitats. Population sizes are highly variable in each generation and $N_e$/N is small due to high fecundity and high juvenile mortality (see
Table 3. Allele frequencies at polymorphic loci for Speyeria nokomis apacheana from the Reese River, central Great Basin.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GPI-I</td>
<td>c</td>
<td>1.000</td>
<td>0.983</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>0.000</td>
<td>0.017</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>MDH-I</td>
<td>b</td>
<td>0.000</td>
<td>0.015</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>1.000</td>
<td>0.985</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>PCD-I</td>
<td>c</td>
<td>1.000</td>
<td>0.989</td>
<td>1.000</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>0.000</td>
<td>0.031</td>
<td>0.000</td>
<td>0.000</td>
<td>0.100</td>
</tr>
<tr>
<td>PGM-I</td>
<td>c</td>
<td>0.810</td>
<td>0.989</td>
<td>0.767</td>
<td>0.806</td>
<td>0.672</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>0.190</td>
<td>0.015</td>
<td>0.233</td>
<td>0.194</td>
<td>0.328</td>
</tr>
<tr>
<td>SOD-I</td>
<td>c</td>
<td>0.647</td>
<td>0.606</td>
<td>0.600</td>
<td>0.556</td>
<td>0.532</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>0.353</td>
<td>0.394</td>
<td>0.400</td>
<td>0.444</td>
<td>0.488</td>
</tr>
</tbody>
</table>

N = 58, 33, 15, 31, 30, 25

Waples 1989). Mean heterozygosity is generally low and is less affected by short-term bottlenecks than is allelic diversity. Low levels of gene flow appear to have restored 4 alleles lost due to drift during the course of the study. Similar trends can be seen with long-term population data for the endangered Uncompahgre fritillary butterfly (Boloria acrocnema) in southwestern Colorado. Population size estimates for 9 of 10 years (1987–1997) at the largest known colony site show that population size fluctuated over 3 orders of magnitude and suggest a long-term N_e/N of 0.278 based on the method of Vucetich et al. (1997; Colorado Natural Heritage Program data). The B. acrocnema population at this site had an estimated mean heterozygosity of 0.031 with 2 moderately polymorphic loci in 1988 (Britten and Brussard 1992). Other insular butterfly species, such as Hesperia dacotae and Euphydryas editha bagis, show similar patterns of population fluctuation and probably very low N_e/N as well (Ehrlich and Murphy 1987, Dana 1991).

The demonstration that fluctuations in population size are the single most important determinant of effective population size (Frankham 1995, 1996, Vucetich et al. 1997) has important implications for insect conservation in general. By contributing to diminished N_e, dramatic fluctuations in population size reduce population genetic variability. It is commonly believed that insects may be sustained with higher genetic diversity than vertebrates. In a review of published estimates, Nei and Graur (1984) found that although 127 invertebrate species had heterozygosities that were on average higher than those of 214 vertebrate species, many invertebrates exhibit heterozygosities lower than vertebrates. The latter situation is clearly the case for butterflies confined to small areas of patchy habitat including Boloria acrocnema and B. improba harryi, Euphydryas editha, and S. n. apacheana (Baughman et al. 1990, Britten and Brussard 1992, Britten et al. 1994b). The erosive effects of drift combined with the demographic threats to persistence exacerbated by small population sizes may compromise the ability of such species to persist and to adapt to rapidly changing environments.

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LITERATURE CITED


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