# Genetic Consequences of Polygyny and Social Structure in an Indian Fruit Bat, *Cynopterus sphinx*. II. Variance in Male Mating Success and Effective Population Size

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# Genetic Consequences of Polygyny and Social Structure in an Indian Fruit Bat, *Cynopterus sphinx.* II. Variance in Male Mating Success and Effective Population Size

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**Abstract**
Variance in reproductive success is a primary determinant of genetically effective population size ($N_e$), and thus has important implications for the role of genetic drift in the evolutionary dynamics of animal taxa characterized by polygynous mating systems. Here we report the results of a study designed to test the hypothesis that polygynous mating results in significantly reduced $N_e$ in an age-structured population. This hypothesis was tested in a natural population of a harem-forming fruit bat, *Cynopterus sphinx* (Chiroptera: Pteropodidae), in western India. The influence of the mating system on the ratio of variance $N_e$ to adult census number (*N*) was assessed using a mathematical model designed for age-structured populations that incorporated demographic and genetic data. Male mating success was assessed by means of direct and indirect paternity analysis using 10-locus microsatellite genotypes of adults and progeny from two consecutive breeding periods ($n = 431$ individually marked bats). Combined results from both analyses were used to infer the effective number of male parents in each breeding period. The relative proportion of successfully reproducing males and the size distribution of paternal sibships comprising each offspring cohort revealed an extremely high within-season variance in male mating success (up to 9.2 times higher than Poisson expectation). The resultant estimate of $N_e/N$ for the *C. sphinx* study population was 0.42. As a result of polygynous mating, the predicted rate of drift (½$N_e$ per generation) was 17.6% higher than expected from a Poisson distribution of male mating success. However, the estimated $N_e/N$ was well within the 0.25–0.75 range expected for age-structured populations under normal demographic conditions. The life-history schedule of *C. sphinx* is characterized by a disproportionately short sexual maturation period scaled to adult life span. Consequently, the influence of polygynous mating on $N_e/N$ is mitigated by the extensive overlap of generations. In *C. sphinx,* turnover of breeding males between seasons ensures a broader sampling of the adult male gamete pool than expected from the variance in mating success within a single breeding period.

**Keywords:** Effective population size, genetic drift, mating system, microsatellite DNA, polygyny, reproductive success, sexual selection

Polygynous mating is one of the most salient features of mammalian social structure and has potentially far-reaching consequences for a diverse array of evolutionary processes (Clutton-Brock 1989, 1991). Variance in male reproductive success is an important determinant of the opportunity for sexual selection (Wade 1979; Wade and Arnold 1980) and genetically effective population size ($N_e$; Wright 1938; Nunney 1993). Polygynous mating primarily affects $N_e$ by reducing the absolute number of breeding males and by skewing the proportional representation of male ancestors in the gene pool of subsequent generations. In populations of many mammalian species, the degree of polygyny may thus exert a powerful influence on the likely course of microevolutionary events.

In populations characterized by polygynous mating and overlapping generations, $N_e$ increases as a positive function of generation interval (Nunney 1993, 1996). This reflects the fact that variance in lifetime reproductive success generally decreases when opportunities for mating are distributed across a greater number of breeding seasons (Clutton-Brock 1988). When generations overlap, the ratio of $N_e$ to adult census number (*N*) is predicted to fall within the range 0.25–0.75 under most demographic circumstances (Nunney 1993, 1996; Nunney and Elam 1994). In species characterized by a relatively rapid maturation period scaled to adult life span, $N_e/N$ exhibits an asymptotic convergence to 0.5 as the generation interval increases (Waite and Parker 1996). According to theory, extreme circumstances are required to reduce $N_e/N < 0.25$ (Nunney 1993, 1996; Nunney and Elam 1994). It remains to be determined whether the extreme variance in male reproductive success thought to characterize populations of harem-forming mammals and lek-mating birds is generally capable of producing such circumstances.

The objective of this study was to test the hypothesis that polygynous mating results in significantly reduced $N_e$ in an age-structured population. This hypothesis was tested in a natural population of the short-nosed fruit bat, *Cynopterus sphinx* (Chiroptera: Pteropodidae), in western India. Using microsatellite genotypes of adults and progeny from consecutive breeding periods, variance in male mating success was inferred from the relative proportion of successfully reproducing males and the size distribution of paternal sibships comprising each offspring cohort. The influence of the mating system on variance $N_e$ was then assessed using a mathematical model designed for age-structured populations that incorporated demographic and genetic data obtained from marked individuals.

*Estimating Effective Sizes of Natural Populations*

Demographic methods for estimating $N_e$ involve making inferences about the potential rate of drift by measuring parameters that influence variance in reproductive success. Although indirect, such methods provide a view of the prospective rate of drift, and can be used to predict the direction and magnitude of changes in $N_e$ that result from variation in ecological or social conditions.

Wright (1931, 1938, 1969) formulated the basic theoretical framework that described how $N_e$ is affected by fluctuating population size, breeding sex ratio, and variance in reproductive success. Hill (1972, 1979) derived the relationship that generalized Wright's results to age-structured populations under conditions of demographic equilibrium (see also Felsenstein 1971; Johnson 1977; Emigh and Pollak 1979). Application of Hill's (1972, 1979) model to natural populations has been hindered by the difficulty of estimating parameters such as the variance and covariance of lifetime reproductive success among adults of both sexes (Wood 1987; Harris and Allendorf 1989). Nunney (1991, 1993, 1996) reformulated the Hill equation for overlapping generations to allow estimation of $N_e$ under the assumption of age-independent survival and fecundity. Insofar as these assumptions remain valid for any particular population, the Hill equation can be expressed in terms of seasonal reproductive parameters and mean generation interval of both sexes. Data from one or several reproductive episodes can thus be used to estimate $N_e$ in lieu of extensive life table data (Nunney and Elam 1994).

## Materials and Methods

### *Study Population*

*Cynopterus sphinx* is a medium-sized (40–70 g) frugivorous bat that is characterized by a harem-forming social structure (Storz and Kunz 1999; Storz et al. 2001a). In western India, adult male *C. sphinx* chew and sever the stems of dense, pendulous flower/fruit clusters of the kitul palm (*Caryota urens*) to create enclosed, bell-shaped roosting spaces called "tents" (Storz et al. 2000b). Tents attract diurnal roosting groups of reproductive females that a single male then defends as a harem. In peninsular India, *C. sphinx* is seasonally polyestrous, having two distinct reproductive periods per year. Parturition typically occurs in February–March and again in June–July (Storz and Kunz 1999; Storz et al. 2000a,b). Females normally give birth to a single pup and can thus produce a maximum of two pups per year (Sandhu 1984).

Fieldwork was conducted in Pune, Maharashtra, India (18°32′N, 73°51′E) over a 25-month period (April 1996–April 1998; Storz et al. 2000b). Within the study area, breeding adults of *C. sphinx* were distributed among nine or 10 diurnal roosting colonies, each of which contained one to five harems and often one or more satellite males in adjacent roosts. The year-round breeding sex ratio of harems averaged 8.9 (females:males), indicating the potential for an extremely high within-season variance in male mating success. The extent to which this potential is realized will depend on the ability of harem males to maintain exclusive mating access to female roostmates. Intermittent transfers among tents by females indicate that territory-holding males may often have simultaneous and sequential contact with a large pool of potential mates in each breeding period (Storz et al. 2000a,b). However, the resultant variance in male mating success within and between seasons can only be reliably determined by means of genetic paternity analysis.

### *Sampling Protocol*

Complete harem groups and solitary male *C. sphinx* were sampled over a period of approximately four weeks immediately following each of two annual parturition periods: July–August 1997 (wet season) and March–May 1998 (dry season). We also sampled all territory-holding males within the study area over a two-year period (1996–1998) that spanned the dates of conception for all sampled offspring (see Storz et al. 2000b). Bats were sampled when nearly all females had given birth, but pups had not been weaned. Collections of every harem included the resident male in addition to known mother-offspring pairs and adult females without suckling young ($n$ = 27 harems). All pups and adults were individually marked and wing-membrane tissue biopsies were taken as a source of DNA for the genetic analysis (Storz et al. 2000a,b, 2001b).

### *Genetic Marker System*

Molecular genetic analysis of parentage and kinship was based on a total of 10 microsatellite DNA markers. Primer sequences, repeat motifs, and polymerase chain reaction protocols were reported previously (Storz 2000; Storz et al. 2001b). Genotypic differentiation between adult males and females was tested using an exact probability $G$-test (Goudet et al. 1996). Using the program FSTAT 2.8 (Goudet 1995), 10,000 genotypic randomizations were used to generate an exact probability distribution under the null hypothesis of no differentiation between the sexes. Polymorphic information content (*PIC*) for each locus was calculated according to the method of Hearne et al. (1992). Given paired maternal-filial genotypes and assuming Hardy-Weinberg equilibrium, the average probability of paternity exclusion for a randomly chosen, unrelated male was calculated according to the method of Jamieson (1994). Expected frequencies of null alleles at each locus were estimated according to the method of Summers and Amos (1996).

### *Estimating Male Mating Success*

Variance in male mating success was estimated using two complementary approaches based on direct and indirect analysis of paternity. Ten-locus microsatellite genotypes of adults and progeny were used to estimate paternity among candidate males (direct analysis) and shared paternity among pups within the same offspring cohort (indirect analysis). In the direct paternity analysis, a total of 185 offspring from each of two consecutive cohorts (1997 wet season and 1998 dry season) were tested against a total of 37 candidate males. The analysis was based on genotypes from a total of 431 individually marked bats. The indirect paternity analysis was based on the same dataset, but excluding adult males. Because patterns of paternal relatedness among offspring within each age cohort can be used to infer the total number of male parents (even when successfully reproducing males remain unsampled), this method provides a population-level estimate of variance in mating success that is unaffected by potential bias in the sampling of candidate males.

Because all pups were matched with known mothers, paternally contributed alleles in offspring could be inferred from paired maternal-filial genotypes. Thus, all tests of pedigree relationship used multilocus paternal haplotypes as the units of analysis, a situation that results in greatly enhanced statistical power (Meagher and Thompson 1986; Thompson and Meagher 1987; Marshall et al. 1998).

*Direct paternity analysis* — Paternity of pups born in the 1997 and 1998 offspring cohorts was assessed by a likelihood-based statistical test using the program CERVUS 1.0 (Marshall et al. 1998). CERVUS uses likelihood ratios as a means of paternity inference when multiple males are not excluded from siring a particular offspring. The likelihood ratio is the likelihood of paternity of a particular male relative to the likelihood of paternity of a randomly chosen male, assuming that genotypic proportions can be predicted from allelic frequencies (i.e., marker loci are in Hardy-Weinberg equilibrium). The natural logarithm of the product of likelihood ratios across multiple, unlinked loci is referred to as a LOD score (Meagher 1986). In CERVUS, paternity assignment is based on a likelihood comparison of alternative father-offspring relationships between the two most likely candidate sires. The test statistic $\Delta$ is defined as the logarithm of the ratio of likelihood ratios, or equivalently, the difference in LOD scores between the two most likely nonexcluded males. CERVUS was used to run a total of 10,000 paternity simulations for the purpose of obtaining critical $\Delta$-values and predicted success rates for paternity assignment at 80% and 95% confidence levels. Each confidence level for paternity assignment was based on the $\Delta$-value surpassed by the corresponding percentage of "true" fathers in the simulations. Each confidence level can be considered a cut-off point for tolerance of false-positive paternity assignments (Type I error). In practice, the 80% confidence criterion is used most frequently for paternity assignment in studies of natural populations (e.g., Coltman et al. 1998, 1999a,b; Rossiter et al. 2000). Using population allelic frequencies from each year separately, simulated offspring genotypes were produced by Mendelian sampling from a pool of randomly generated parental genotypes. The simulated data were generated under the assumption that 70% of potentially breeding males remained unsampled, and that single-locus mismatches between parent and offspring (due to scoring error or germline mutation) occurred at a frequency of 0.005.

*Indirect paternity analysis* — The frequency of shared paternity within each offspring cohort was assessed by identifying groups of at least two paternal half-siblings. Thus, the proportional representation of successfully reproducing males (whether sampled or not) could be inferred from the size distribution of paternal sibships comprising each cohort. The assessment of paternal relatedness also provided a means of cross-validating the results of the direct paternity analysis for pups that were allegedly sired by sampled males. Paternal half-siblings were identified by means of a likelihood-based statistical test using the program KINSHIP 1.3 (Goodnight and Queller 1999). For a given pair of individuals, hypotheses about particular pedigree relationships can be expressed by the probability that shared alleles are identical by paternal or maternal descent ($R_p$ and $R_m$, respectively). To test for shared paternity among pairs of offspring born to different mothers, the primary hypothesis was that paternally derived alleles were identical by direct descent from a common father ($R_p = 0.5$, $R_m = 0$). The null hypothesis was that alleles were not identical by descent through either route of Mendelian gene transmission ($R_p = 0$, $R_m = 0$). Probability calculations were weighted according to the ambient level of allele-sharing in the total population and incorporated a bias correction for group membership, following the method of Queller and Goodnight (1989). Pups were identified as paternal half-siblings on the basis of LOD scores, calculated as the (base-10) log-likelihood ratio of the two hypotheses (primary/null). Statistical significance of LOD scores was assessed by means of computer simulation, as implemented in KINSHIP. Using allelic frequencies from adults and progeny in each year separately, the simulation routine was used to generate 10,000 pairs of multilocus genotypes conforming to the pedigree relationships specified by the primary and null hypotheses. The simulated data were then used to determine the critical value of the likelihood ratio needed to reject the null hypothesis at a 95% confidence level. In cases where shared paternity was indicated for more than two individuals, pups were included in a common sibship only if more than 80% of the total number of pairwise LOD scores surpassed the 95% confidence level. Thus, inclusion in a putative sibship of fewer than five pups required that all pairwise LOD scores exceed the value obtained by 95% of true paternal half-siblings in the simulations.

### *Estimating Variance in Male Mating Success*

Offspring sired by sampled males were assigned to sibships on the basis of results of the direct paternity analysis. Similarly, offspring sired by unsampled males were assigned to sibships on the basis of paternal relatedness. The total number of paternal sibships thus provided an estimate of the total number of male parents represented in each offspring cohort. Variance in male mating success per breeding period was estimated from the size distribution of paternal sibships. Accordingly,

$$\sigma^2 = \frac{N_m \sum_i p_i^2 - \left(\sum_i p_i\right)^2}{N_m^2}, \quad (1)$$

where $N_m$ is the total number of sexually mature males in the population (including nonbreeding males) and $p_i$ is the number of pups in the $i$th paternal sibship. The standardized variance in male mating success ($I_{bm}$) was calculated as

$$I_{bm} = \frac{N_m \sum_i p_i^2 - \left(\sum_i p_i\right)^2}{n^2}, \quad (2)$$

where $n$ is the total number of pups in the offspring cohort. In any given breeding period, there is variance in progeny number among successful males in addition to the variance between successful and unsuccessful males that results when ($N_m - s$) of the males do not mate (where $s$ is the number of paternal sibships).

*Estimating $N_e/N$*

The ratio of effective size to adult census number ($N_e/N$) for the *C. sphinx* study population was estimated according to the method of Nunney (1993, equation A2):

$$N_e/N = [4r(1-r)T] \div \{[A_m(1-r) + A_f r] + [I_{bm}(1-r) + I_{bf} r] + [A_m I_{Am}(1-r) + A_f I_{Af} r]\}, \quad (3)$$

where $r$ is the operational sex ratio (expressed as the proportional number of sexually mature males), $T$ is the mean generation interval ( = $[T_m + T_f]/2$, where $T_i$ is the generation interval of sex $i$), $A_i$ is the average adult lifespan of both sexes, $I_{Ai}$ is the standardized variance in adult lifespan of both sexes, $I_{bf}$ is the standardized variance in female fecundity per breeding period, and $I_{bm}$ is the standardized variance in male mating success per breeding period. All parameters were estimated using demographic and behavioral data from Storz et al. (2000b), in conjunction with information on ontogeny and reproduction from Storz and Kunz (1999). To parameterize the model, the estimate of time to reproductive maturity of sex $i$ ($M_i$) was averaged across each of the two annual cohorts of offspring. Annual adult survivorship ($v_i$) was assumed to be equal for males and females. Estimation of the average adult life span ($A_i$) was based on the assumption of age-independent survivorship (Nunney 1993; Nunney and Elam 1994), whereby $A_i = 1/(1 - v_i)$. Estimation of generation interval ($T_i$) was based on the assumption of age-independent fecundity, whereby $T_i = M_i - 1 + A_i$ (Nunney 1996; Nunney and Elam 1994). Estimates of the operational sex ratio ($r$) were obtained from census counts of the entire roosting population of adult *C. sphinx* within the study area (including nonterritorial males), averaged across seasons (see Storz et al. 2000a,b). Estimates of the seasonal mean and variance of female reproductive success were based on the number of progeny raised to weaning age. Following the method outlined by Nunney and Elam (1994, pp. 183–184), the standardized variance in female fecundity ($I_{bf}$) was estimated as the seasonal variance in progeny number in excess of Poisson expectation. Estimates of the standardized variance in seasonal reproductive success for both sexes were averaged across the two annual breeding periods.

## Results

The exact probability *G*-test revealed no statistically significant differences in genotypic frequencies between the sexes ($P = 0.221$). Genotypic data from all sampled adults were therefore pooled for the purpose of estimating allelic frequencies. In the pooled sample of breeding adults from both seasons, marker loci exhibited no statistically significant departures from allelic or genotypic equilibria (Storz et al. 2001b). No mismatches were observed between mother and pup genotypes at any locus, and indirect tests based on allelic and genotypic frequencies indicated that null alleles were either nonexistent or present at negligible frequencies (Table 1). High levels of heterozygosity and allelic diversity resulted in a cumulative probability of 0.999 for random paternity exclusion (Table 1).

**Table 1.** Summary statistics for microsatellite DNA markers used in the assessment of parentage and kinship in the study population of short-nosed fruit bats, *Cynopterus sphinx*. Locus 3 is a dinucleotide repeat, CSP-1 through CSP-3 are trinucleotide repeats, and CSP-4 through CSP-9 are tetranucleotide repeats (Storz 2000; Storz et al. 2001b). Number of alleles per locus averaged 10.3 (range = 5–17; Storz et al. 2001b). *N*, number of bats genotyped per locus; $f_0$, expected frequency of null alleles; *PIC*, polymorphic information content; and $P_1$, probability of paternity exclusion.

| Locus | *N* | $f_0$ | *PIC* | $P_1$ |
|---|---|---|---|---|
| Locus 3 | 431 | 0.011 | 0.72 | 0.546 |
| CSP-1 | 431 | −0.016 | 0.69 | 0.514 |
| CSP-2 | 431 | −0.022 | 0.70 | 0.511 |
| CSP-3 | 431 | 0.019 | 0.34 | 0.189 |
| CSP-4 | 431 | −0.008 | 0.74 | 0.563 |
| CSP-5 | 431 | 0.001 | 0.69 | 0.499 |
| CSP-6 | 431 | 0.026 | 0.84 | 0.706 |
| CSP-7 | 431 | 0.001 | 0.79 | 0.642 |
| CSP-8 | 420 | −0.011 | 0.72 | 0.566 |
| CSP-9 | 431 | −0.018 | 0.43 | 0.253 |

*Male Mating Success*

*Direct paternity analysis* — Using the 80% confidence level as the minimum criterion for paternity assignment, the direct analysis revealed that 118 of 185 pups (64%) were sired by territory-holding candidate males included in our sample. In both the 1997 and 1998 offspring cohorts, the majority of paternity assignments were made at the 95% confidence level. The number of paternity assignments in each cohort greatly surpassed those predicted by the simulations. At the 80% confidence level, observed success rates for paternity assignment were 81% in the 1997 offspring cohort (vs. 35% expected) and 54% in the 1998 offspring cohort (vs. 37% expected). This indicates that the majority of true fathers were included in our sample of candidate males. In 14 of 23 cases where paternity was assigned at a confidence level between 80% and 95%, the most likely male was not excluded at any loci, and the next most likely male was excluded at one or more loci. The remaining nine cases involved genotypic mismatches between putative father-offspring dyads at no more than a single locus. Use of the 80% confidence level for paternity assignment was validated by an independent assessment of paternal relatedness among pups identified as offspring of the same male (see below). A higher proportion of pups was sired by sampled males in the 1997 offspring cohort (54/67, 81%) relative to the 1998 cohort (64/118, 54%). Moreover, a far greater proportion of pups in the 1997 cohort were sired by males that were resident in the natal colony at the time of parturition (Figure 1). In the 1997 cohort, 52 of 54 paternities (96%) were assigned to territory-holding males that were resident in the natal colony. In the 1998 cohort, however, only 25 of 64 paternities (39%) were assigned to resident males. Extending the paternity analysis across both offspring cohorts revealed that three of the sampled males sired pups in consecutive breeding periods.

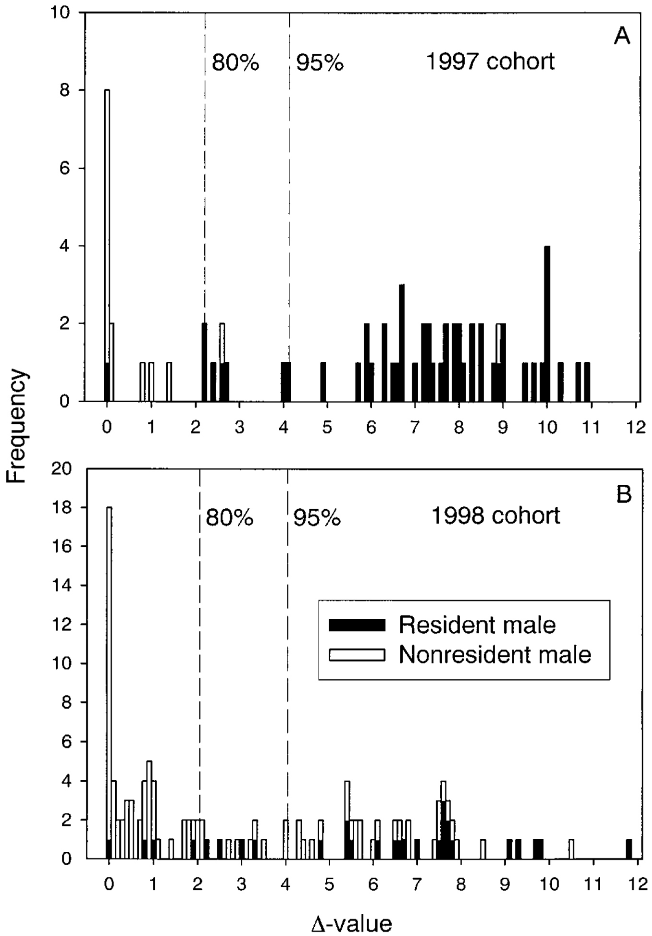


**Figure 1.** Distribution of Δ-values of most likely candidate males in the (A) 1997 and (B) 1998 offspring cohorts. Dotted lines denote critical Δ-values for paternity assignment at 80% and 95% confidence levels. Resident males are adult males that held territories within the natal colony of a particular offspring at the time of parturition

*Indirect paternity analysis.* — Using the 95% confidence level for identifying pups as paternal half-siblings, and using the "80% rule" for assigning membership to any given sibship, a total of 15 paternal sibships were identified in the 1997 offspring cohort, and 54 in the 1998 cohort. Sibships comprising at least two pups accounted for 91% of the total number of offspring in the 1997 cohort, and 78% in the 1998 cohort. In all cases, pups that were implicated as paternal half-siblings by the direct paternity analysis were also assigned to a common sibship on the basis of paternal allele sharing alone. There were no cases in which a pup with unassigned paternity was included in a sibship composed of pups that were sired by a sampled male. Likewise, pups that were sired by the same sampled male were never assigned to separate sibships. In addition to validating the paternity assignments of sampled males, the analysis of paternal relatedness using KINSHIP also identified many sibships of at least two pups that were sired by unsampled males ($n = 2$ in 1997, and $n = 16$ in 1998). Extending the analysis across both years, shared paternity between consecutive cohorts was identified for a total of 57 offspring comprising six paternal sibships (size range = 2–31 offspring). Three of the between-year sibships were sired by sampled males, as revealed by the direct paternity analysis (see above). Among the limited number of recaptured females that produced pups in consecutive breeding periods, two of 16 pairs of maternal siblings were sired by the same male. Thus, the mating system of *C. sphinx* results in the production of large numbers of paternal half-siblings within age cohorts, but relatively few full-siblings between consecutive cohorts.

**Table 2.** Summary statistics for estimates of male mating success in the 1997 wet-season and 1998 dry-season offspring cohorts

| Cohort year | No. of pups | No. of sires [1] | Max. no. of progeny per male | Mean no. of progeny per male | Variance in male mating success | Standardized variance ($I_{bm}$) |
|---|---|---|---|---|---|---|
| 1997 | 67 | 15 (9) | 20 | 0.79 | 7.22 | 11.73 |
| 1998 | 118 | 54 (20) | 11 | 0.75 | 2.27 | 4.04 |
| Combined | 185 | 63 (25) | 31 | 1.52 | 11.05 | 4.76 |

[1] Number of candidate males identified as sires of one or more offspring is given in parentheses.

*Variance in Male Mating Success and $N_e/N$*

The study population of *C. sphinx* was characterized by a high variance in male mating success, as indicated by the relative proportion of successfully reproducing males and the size distribution of paternal sibships comprising each offspring cohort. The 1997 offspring cohort was sired by a total of 15 males (nine of which were sampled), and the 1998 cohort was sired by a total of 54 males (20 of which were sampled; Table 2). Because a total of six males sired pups in both cohorts, the entire collection of 185 offspring was sired by a total of 63 males (25 of which were sampled). Paternal sibships sired by sampled males were generally larger than those sired by unsampled males (Mann-Whitney *U*-test, 1997 cohort: $U_{n1:n2} = 4.5_{7:8}$, $P = 0.005$; 1998 cohort: $U_{n1:n2} = 231.5_{20:34}$, $P = 0.036$). Seasonal variance in male mating success was 9.2 times higher than Poisson expectation in 1997 and 3.0 times higher in 1998. The standardized variance in male mating success ($I_{bm}$) was 2.9 times higher in the 1997 offspring cohort than in the 1998 cohort (Figure 2). The average within-season $I_{bm}$ (7.85) was 1.7 times higher than the estimated $I_{bm}$ across consecutive breeding periods.

In conjunction with estimates of parameters describing reproduction and demography of the *C. sphinx* study population, substitution of the average within-season $I_{bm}$ into equation (3) resulted in an $N_e/N$ estimate of 0.42 (Table 3). In contrast, substitution of the Poisson-expected value of $I_{bm}$ resulted in an $N_e/N$ estimate of 0.51. Thus, as a result of polygynous mating, the predicted rate of drift (½$N_e$ per generation) in the *C. sphinx* study population was 17.6% higher than expected from a Poisson distribution of male mating success.

## Discussion

*Variance in Mating Success, Overlapping Generations, and $N_e/N$*

Male competition for mating access to reproductive females (or female preference for some males over others) results

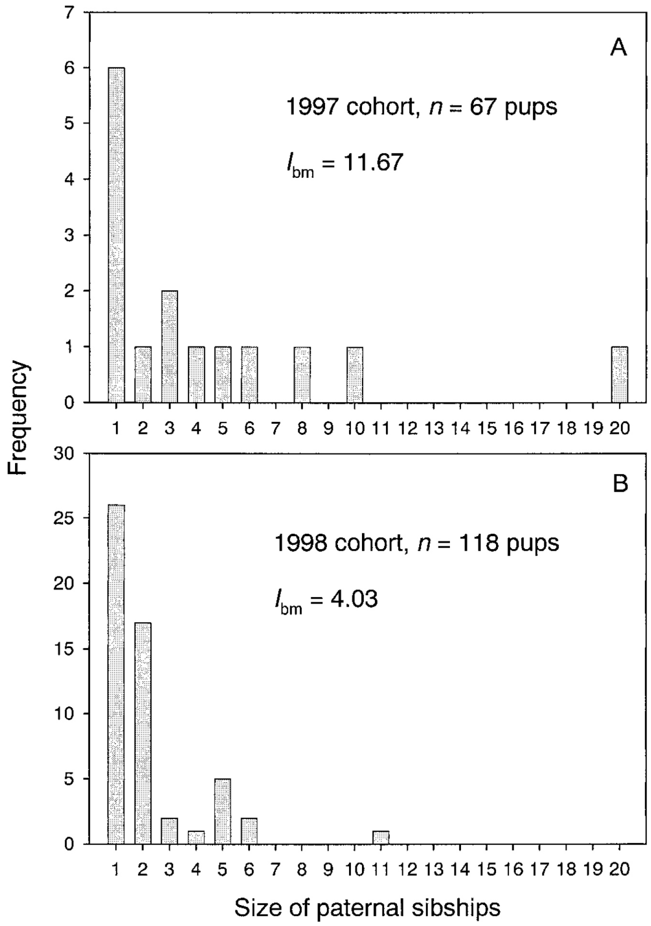


**Figure 2.** Size distribution of paternal sibships in the (A) 1997 and (B) 1998 offspring cohorts. A sibship of size one represents a pup with no shared paternity in the same age cohort

in a nonrandom sampling of the adult male gamete pool in each generation, thereby increasing the rate of genetic drift. We quantified the effect of this sampling process in a natural population using a molecular-genetic assessment of paternity. The results indicate that the *C. sphinx* study population is characterized by an extremely high within-season variance in male mating success, as expected from the harem-forming mode of social structure (Storz et al. 2000a,b). The estimated $N_e/N$ for the *C. sphinx* study population was substantially lower than would be expected if a more egalitarian mating system prevailed. However, despite the high within-season variance in male mating success, the estimated $N_e/N$ was well within the 0.25–0.75 range expected for age-structured populations under otherwise unexceptional demographic conditions (Nunney 1993, 1996; Nunney and Elam 1994). The life-history schedule of *C. sphinx* (and bats in general) is characterized by a disproportionately short sexual maturation period scaled to adult life span. Consequently, the influence of polygynous mating on $N_e/N$ is mitigated by the extensive overlap of generations (Nunney 1993; Waite and Parker 1996). In *C. sphinx,* as in other long-lived, polygynous mammals (Clutton-Brock et al. 1988; Altmann et al. 1996), continual turnover of breeding males ensures a broader sampling of the adult male gamete pool than indicated by the disproportionate posterity of top-ranking individuals within a single season.

**Table 3.** Parameters (means and standardized variances) that describe reproduction and demography in a local population of *Cynopterus sphinx* in Pune, India. The parameter estimates were used to calculate $N_e/N$ from the model of Nunney (1993, equation A2). Given overlapping generations, this model provides an estimate of $N_e/N$ based on the assumption of age-independent survival and fecundity and nonheritable reproductive success. Subscripts of parameter symbols denote values for females (f) and males (m). All units of time are indexed relative to the annual breeding cycle of *C. sphinx.* See text for computational details

| Parameter | Symbol | Mean/ variance |
|---|---|---|
| Average time to sexual maturity | $M_f$ | 0.54 |
| | $M_m$ | 1.46 |
| Annual adult survivorship [1] | $v_f$ | 0.88 |
| | $v_m$ | 0.88 |
| Average adult life span [1] | $A_f$ | 8.33 |
| | $A_m$ | 8.33 |
| Average generation interval | $T_f$ | 7.88 |
| | $T_m$ | 8.79 |
| Standardized variance in female reproductive success (per breeding period) | $I_{bf}$ | 0.13 |
| Standardized variance in male reproductive success (per breeding period) [2] | $I_{bm}$ | 7.85 |
| Breeding sex ratio (proportion of males) [2] | $r$ | 0.47 |
| Adult census number [2] | $N$ | 257.5 |
| Effective population size/adult census number | $N_e/N$ | 0.42 |

[1] Annual survivorship of males (and thus average adult life span) was assumed to be equal to that of females.
[2] Average values for two consecutive breeding periods (1997 wet season and 1998 dry season).

The results of this study underscore several important issues about methods used to estimate the effective size of age-structured populations. When generations overlap, the impact of polygynous mating on $N_e/N$ is typically much diminished relative to expectations based on discrete-generation formulas. The problem with applying such formulas to species with overlapping generations is that an enumeration of breeding adults at any one point in time ignores the possibility that individuals failing to reproduce in one breeding period may have reproduced previously and may reproduce in the future. The demographic model of Nunney (1993; see also Nunney and Elam 1994) accounts for temporal changes in the reservoir of nonreproductive adults by using seasonal estimates of $I_{bm}$ and $I_{bf}$ in conjunction with an estimate of the sex ratio derived from the total adult census number. Although Nunney's (1993) model accounts for the potential disparity between seasonal and lifetime variance in reproductive success (provided that sex ratio is estimated in an appropriate manner), the use of cross-sectional data may be biased if there is substantial interannual variation in mating patterns. For example, long-term studies of free-ranging red deer (*Cervus elaphus*) and Soay sheep (*Ovis aries*) have demonstrated cohort-specific effects on male reproductive success as a result of environmental and demographic variation in the year of birth (Pemberton et al. 1996, 1999; Clutton-Brock et al. 1997; Rose et al. 1998; Coltman et al. 1999b).

In the *C. sphinx* study population, the distribution of paternity was more highly skewed in the 1997 (wet-season) cohort of offspring than in the 1998 (dry-season) cohort. Differences in the degree of polygyny between the two offspring cohorts are primarily attributable to seasonal variation in the dispersion of females. Tight clustering of females in diurnal roosts appears to facilitate a male mating strategy of resource-defense polygyny (Storz et al. 2000a,b). When aggregations of reproductive females are distributed among a limited number of roosts that males can defend as territories, a small fraction of the adult male population will likely succeed in monopolizing opportunities for mating. In the dry season, female dispersion is highly clumped and average harem size is 2.3 times higher than in the wet season (Storz et al. 2000b). Pups born in the wet season are conceived 115–135 days prior, during the postpartum estrus period (midway through the dry season) when the potential for polygyny is greatest (Storz and Kunz 1999; Storz et al. 2000a,b). The proportionally greater degree of polygyny reflected in the wet-season offspring cohort was therefore consistent with seasonal differences in average size of harems. These seasonal differences in female dispersion (and thus potential for polygyny) are consistent on a year-to-year basis (Storz et al. 2000b).

There are also some important methodological issues related to the estimation of $I_{bm}$ that should be considered. With some exceptions (e.g., Waite and Parker 1997; Bouteiller and Perrin 2000), most estimates $N_e/N$ for natural populations have been based on untested assumptions about genetic consequences of the social (apparent) mating system. Although analysis of parentage based on molecular markers can potentially provide highly refined estimates of $I_{bm}$, there are several issues that need to be considered with regard to sampling design. As illustrated by the results of several recent marker-based studies of male mating success in polygynous mammals, even the most comprehensive sampling efforts typically leave the vast majority of offspring with unidentified sires (Coltman et al. 1998; Worthington-Wilmer et al. 1999). When positive paternity assignments are restricted to a nonexhaustive sample of candidate males, the resultant ascertainment bias may render $I_{bm}$ estimates meaningless as population-level descriptors of variance in reproductive success. The approach used in this study was based on the sampling of paternal alleles in an exhaustive sample of pups with known mothers, thereby avoiding problems of sampling bias with regard to candidate males. This approach for estimating $I_{bm}$ should greatly facilitate estimation of $N_e/N$ for natural populations.

### *Demographic Determinants of $N_e/N$*

It is important to note that the analysis presented here provides a static estimate of $N_e/N$; temporal fluctuations in population size could potentially alter the ratio substantially. The impact of fluctuating population size could be factored into an estimate of $N_e/N$ by substituting the harmonic mean value of $N$ (Wright 1938; Motro and Thomson 1982; Lande and Barrowclough 1987) or the standard deviation of log-transformed $N$ through time (Vucetich et al. 1997). Predictive models based on constant $N$ may provide a reasonable estimate of short-term $N_e/N$, but fluctuating population size may often reduce long-term $N_e/N$ to levels below the range of theoretical expectation. Indeed, Frankham (1995) demonstrated that the effects of fluctuating population size accounted for the greatest fraction of variance in empirical estimates of $N_e/N$ across a diverse array of animal taxa. Reanalysis of $N_e/N$ ratios that factored in the effects of fluctuating population size successfully reconciled theoretical expectations with empirical estimates of $N_e/N \ll 0.25$ (Vucetich et al. 1997). Regardless of how static estimates of $N_e/N$ translate into long-term rates of drift, the approach used in this study provides a straightforward means of quantifying the relative importance of reproductive strategies and life-history traits as determinants of $N_e$. Integrating the effects of fluctuating population size into such an assessment would require long-term data on the interaction between population density and variance in reproductive success (e.g., Pemberton et al. 1996, 1999; Clutton-Brock et al. 1997; Rose et al. 1998; Coltman et al. 1999b).

Inbreeding represents another avenue by which variance in reproductive success of individuals (or alleles) can influence $N_e$. This can occur as a result of nonrandom mating in a single panmictic population (Caballero and Hill 1992) or as a result of subdivision of the population into a network of local demes that are interconnected by varying levels of gene flow (Whitlock and Barton 1997; Nunney 1999; Wang and Caballero 1999). The latter scenario is more appropriate when considering the distribution of genetic variation in mammalian populations that are subdivided into socially defined breeding units (Storz 1999). The adult population of *C. sphinx* exhibited no strong or consistent departures from panmictic expectations (Storz et al. 2001b), indicating that social structure does not promote levels of inbreeding or genetic subdivision that would be expected to affect $N_e$ of the total population (Nunney 1999). Consequently, there is no evidence to suggest that our estimate of $N_e/N$ for the *C. sphinx* study population is confounded by inbreeding or population substructure.

With regard to seasonal variance in female fecundity, our estimate of $I_{bf}$ for the *C. sphinx* study population (0.13) was just below the lower range of similar estimates for birds and mammals (0.14–0.64) that were based on annual numbers of fledglings or weaned young, respectively (Nunney 1996). In group-living social mammals, several factors have the potential to increase variance in female progeny production to levels well above Poisson expectation. For example, reproductive suppression of social subordinates in cooperatively breeding groups may dramatically increase variance in female fecundity. Also, in many social species the size of the breeding group to which an individual belongs can affect reproductive success (Armitage 1986; Clutton-Brock et al. 1988; Vehrencamp et al. 1988). In *C. sphinx,* however, there is no evidence for reproductive suppression within groups (Storz et al. 2000b).

### *Assumptions of the Model*

The model used here to estimate $N_e/N$ also assumes that reproductive success is age-independent and nonheritable (Nunney 1993, 1996; Nunney and Elam 1994). With regard to the first assumption, age-specific variation in male mating success has been documented in several polygynous mammals (e.g., Clutton-Brock et al. 1988; Coltman et al. 1999a,b). However, among *C. sphinx* males in the study population, there was no evidence that harem acquisition or mating success were strongly age dependent (Storz et al. 2000b; J. F. Storz, unpubl. data). When younger males are excluded from breeding, age specific effects on $I_{bm}$ can be easily accommodated in Nunney's (1993) model by recognizing that males are, in effect, taking longer to attain sexual maturity (and the estimate for $M_m$ is increased accordingly; Nunney 1996). Heritability of fitness can potentially reduce $N_e/N$ by producing correlated changes in allelic frequencies across consecutive generations. In group-living social mammals, correlations in fitness across generations may often occur when newly recruited offspring inherit parental territories or breeding status. In *C. sphinx*, however, juveniles of both sexes disperse from their natal harem before attaining sexual maturity (Storz et al. 2000b), so there would seem to be little scope for such effects.

*Mammalian Mating Systems, Life History, and $N_e/N$*

Our estimate of $N_e/N$ for the *C. sphinx* study population may be generally applicable to a large number of phyllostomid and pteropodid bat species, most of which are characterized by polygynous mating systems and overlapping generations (Wilkinson 1987; Fleming 1988). Our estimate of $N_e/N$ for *C. sphinx* was considerably lower than similar estimates for nine other mammalian species obtained by the same method (median = 0.72, range = 0.56–1.27; Nunney and Elam 1994; Bouteiller and Perrin 2000). Relative to other mammalian taxa, bats may be characterized by generally low $N_e/N$ ratios due to a disproportionately short sexual maturation period scaled to adult life span. However, compared to other mammalian orders, bats do not exhibit levels of genetic heterozygosity or karyotypic diversity consistent with long-term small $N_e$-values (Bush et al. 1977; Coyne 1984). Among mammalian taxa characterized by an extensive overlap of generations, variation in long-term $N_e$ may have little to do with differences in mating systems. Instead, rates of drift over evolutionary time scales are likely highest in lineages characterized by stochastic variation in population numbers.

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