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Evolutionary Dynamics of the Short-Nosed Fruit Bat, *Cynopterus sphinx* (Pteropodidae): Inferences from the Spatial Scale of Genetic and Phenotypic Differentiation

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We report the results of a population-genetic study of the short-nosed fruit bat, *Cynopterus sphinx* (Pteropodidae). The purpose of our study was to assess the relative importance of drift, gene flow, and spatially varying selection in shaping patterns of genetic and phenotypic variation across a latitudinal climatic gradient in peninsular India. At a microgeographic scale, polygynous mating resulted in a substantial reduction of effective population size. However, at a macrogeographic scale, rates of migration were sufficiently high to prevent a pronounced degree of stochastic differentiation via drift. Spatial analysis of genetic and phenotypic differentiation revealed that clinal variation in body size of *C. sphinx* cannot be explained by a neutral model of isolation by distance. The geographic patterning of morphometric variation is most likely attributable to spatially varying selection and/or the direct influence of latitudinally ordered environmental effects. The combined analysis of genetic and phenotypic variation indicates that recognized subspecies of *C. sphinx* in peninsular India represent arbitrary subdivisions of a continuous spectrum of clinal size variation.

Introduction

What is the relative importance of drift versus gene flow in driving the genetic differentiation of partially isolated populations? What is the relative importance of spatially varying selection versus stochastic processes in maintaining clinal variation in phenotypic traits? These questions are of central importance to our understanding of local adaptation and the determinants of

geographic variation (Endler, 1977; García-Ramos and Kirkpatrick, 1997; Haldane, 1948; Slatkin, 1973, 1978). Many key inferences about the role of selection in maintaining clinal variation have been obtained by relating inferred dispersal distances to cline widths (Barton and Gale, 1993). Highly vagile animals such as bats and birds are of particular interest in this regard because the persistence of clinal variation requires recurrent selection to counterbalance the homogenizing effect of gene flow. For example, cline widths of approximately 50 km have been documented for Robertsonian fusions between populations of the phyllostomid bat *Uroderma bilobatum* in Central America (Baker, 1981). The standard deviation of single-generation dispersal distances was estimated to be 11 km, indicating that stable maintenance of the observed cline would require selection coefficients of >0.37 against heterozygotes in the contact zone (Barton, 1982).

Relative to the effects of selection, it is considerably more difficult to quantify the importance of drift as a cause of spatial differentiation. In a review of empirical data on cline widths in mammalian populations, Barton (1990) suggested that spatial patterns of differentiation may be greatly affected by nonrandom breeding and drift at the local population level. Similarly, Patton and Smith (1990) argued that the extreme levels of genetic and phenotypic differentiation observed in North American pocket gophers (genus *Thomomys*) are largely attributable to the localized effects of sampling drift caused by polygynous mating and extinction-recolonization dynamics. Since pocket gophers are characterized by highly restricted dispersal capabilities, stochastic fluctuations in allelic frequencies at a microspatial scale may be an important determinant of broad-scale population genetic structure (Daly and Patton, 1990; Patton and Feder, 1981; Patton and Yang, 1977).

Measuring rates of drift requires estimates of effective population size (N_e). It is therefore important to determine whether estimates of N_e for local populations can be used to predict macrogeographic patterns of genetic differentiation. This is relevant to the broader issue of whether local population processes can be extrapolated to broad-scale patterns of genetic differentiation (Lidicker and Patton, 1987).

Here we summarize an analysis of genetic and phenotypic variation in the short-nosed fruit bat, *Cynopterus sphinx*, across a range of spatial scales in peninsular India. We start by examining the influence of polygynous mating and overlapping generations on N_e. We then assess the extent to which estimates of N_e at the local population level can inform the analysis of genetic differentiation at a broader geographic scale. After characterizing the geographic patterning of neutral genetic variation, we compare relative levels of genetic and phenotypic differentiation to assess the possible role of spatially varying selection in maintaining a latitudinal cline in body size. Specifically, we compare relative levels of between-population divergence in body size and neutral DNA markers to assess whether the observed pattern of clinal size variation can be explained by a neutral model of isolation by distance.

Natural History

Cynopterus sphinx is a harem-forming frugivorous bat that is widely distributed across the Indomalayan Region (Storz and Kunz, 1999). In one of our main study sites in western India (Pune, Maharashtra, 18°32′ N, 73°51′ E), harem breeding groups of *C. sphinx* roost in tents constructed within flower/fruit clusters of the kitui palm (*Caryota urens*; Storz *et al.*, 2000b). The breeding population of *C. sphinx* in Pune is subdivided into diurnal roosting colonies, each containing one to five harems and often one or more satellite males in adjacent roosts. Colonies comprise all bats occupying flower/fruit cluster tents in a single tree, or cluster of two or three adjacent trees (Figure 1). In many cases, there is only one tent-roosting harem per colony. In

Figure 1. (a) A kitui palm tree *Caryota urens* that housed a colony of *Cynopterus sphinx* in Pune, India. Three flower/fruit clusters that were modified as tents (T3, T4, and T5) and occupied by bats in the 1997 wet season are indicated. Harems of *C. sphinx* roosting in the altered crowns of mature fruit clusters (tent T4 and tent T5) in the 1998 dry season, are shown in **(b)** and **(c)**, respectively. The harem males in each tent are indicated by arrows.
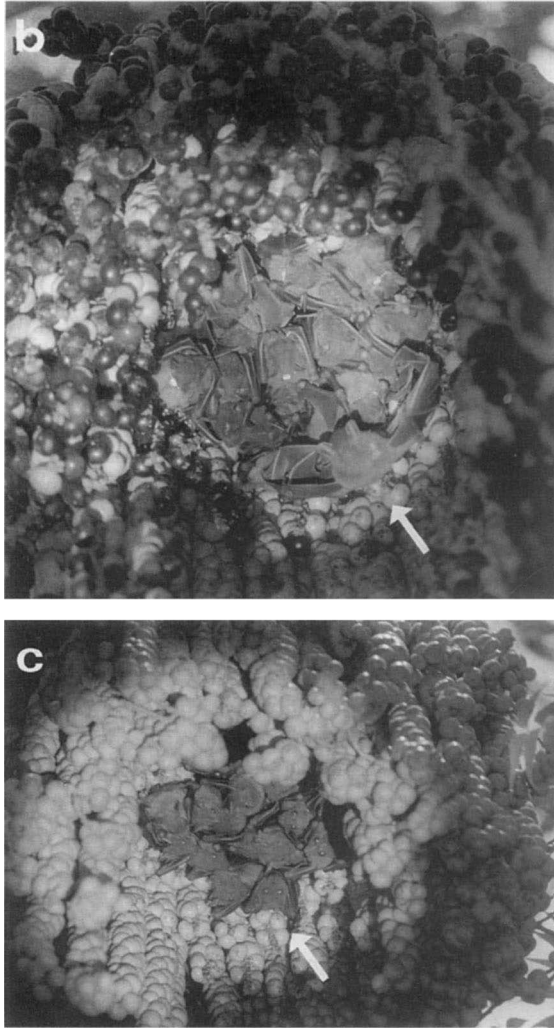
Figure 1, continued.

other colonies, several harems occupy different tents in the same or adjacent trees. Harems consist of a single adult male roosting in association with one to 37 reproductive females and their dependent young. Harem size averages 6.1 adults in the wet season (SD = 3.5) and 13.6 adults in the dry season (SD = 8.5). The same harem social configuration is maintained year-round, despite a high degree of synchrony and seasonably in the timing of reproduction. Adult females often remain associated as roost-mates from one parturition period to the next, and group cohesion is unaffected by turnover of harem males. Juveniles of both sexes disperse after weaning and sexually immature bats are never present in harems at the time of parturition (Storz *et al.*, 2000a,b).

The Local Population Level: Genetic Consequences of Polygyny

In animal taxa characterized by polygynous mating systems, variance in male reproductive success is a primary determinant of N_e (Nunney, 1993; Wright, 1938) and 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). When generations overlap, the ratio of N_e to adult census number (N) is predicted to fall within the range of 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 lifespan, N_e/N exhibits an asymptotic convergence to 0.5 with increasing generation time (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 high variance in male reproductive success thought to characterize populations of harem-forming mammals and lek-mating birds is generally capable of producing such circumstances.

One of the objectives of our study of *C. sphinx* was to test the hypothesis that polygynous mating results in a significantly reduced N_e in a population with overlapping generations. This hypothesis was tested in a natural population of *C. sphinx* in Pune, India. Using 10-locus microsatellite genotypes of adults and progeny from consecutive breeding periods (Storz *et al.*, 2001b,c), 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 N_e was then assessed using a model designed for age-structured populations that incorporated demographic and genetic data obtained from individually marked bats.

Same-age offspring were assigned to sibships using a likelihood-based analysis of paternal relatedness (Storz *et al.*, 2001c). Within-season variance in male mating success was then estimated from the size distribution of paternal sibships comprising each offspring cohort. Accordingly,

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

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

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

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).

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

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

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 (for details of parameter estimation, see Storz *et al.*, 2001c).

Results of our analysis indicated that the study population of *C. sphinx* was characterized by an extremely high variance in male mating success (Figure 2), as expected from its harem-forming mode of social organization (Storz *et al.*, 2000a,b). 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 were primarily attributable to seasonal variation in the dispersion of females. Tight clustering of females in diurnal roosts facilitates a male mating strategy of resource-defense polygyny (Storz *et al.*, 2000a,b). When aggre-

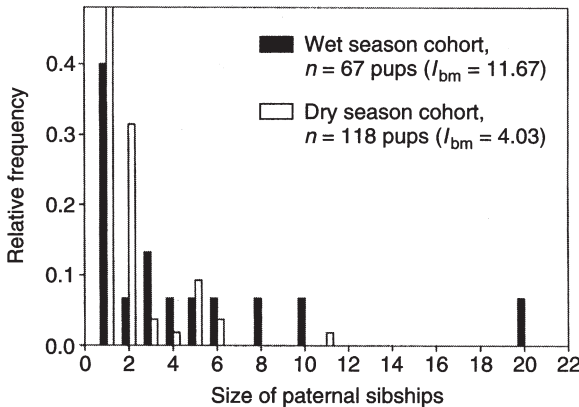

Figure 2. Size distribution of paternal sibships in two consecutive offspring cohorts (wet season and dry season) of *C. sphinx* in Pune, India. A "sibship" of size 1 represents a pup with no shared paternity in the same age cohort.

gations 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-fold higher than in the wet season. Pups born in the wet season are conceived 115-135 days previously, during the post-partum estrus period (midway through the dry season) when the potential for polygyny is greatest (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 (Storz et al., 2001b).

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. By 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. 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 that of bats in general) is characterized by a disproportionately short sexual maturation period scaled to adult lifespan. 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, continual turnover of breeding males ultimately ensures a broader sampling of the adult male gamete pool than indicated by the disproportionate posterity of top-ranking individuals within a single season.

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 (McCracken and Wilkinson, 2000; Wilkinson, 1987). Our estimate of N_e/N for *C. sphinx* is considerably lower than similar estimates obtained for 10 other mammalian species (median = 0.66, range =0.51–1.27; Table 1). Consideration of lineage-specific life-history schedules suggests that bats may be characterized by generally low N_e/N ratios relative to other mammalian taxa (Storz et al., 2001c). However, compared with other orders of mammals, 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

Table 1. Demographic Estimates of the Ratio of Variance Effective Size to Adult Census Number (N_e/N) for Populations of 11 Mammalian Species

Species	N_e/N	T_m, T_f	References
White-toothed shrew, *Crocidura russula*	0.60	1.0, 1.0	Bouteiller and Perrin, 2000
Short-nosed fruit bat, *Cynopterus sphinx*	0.42	8.79, 7.88	Storz et al., 2001c
Human (Gainj), *Homo sapiens*	1.27	38.3, 31.3	Storz et al., 2001d
Savannah baboon, *Papio cynocephalus*	0.51	10.1, 10.2	Storz et al., 2002
Grizzly bear, *Ursus arctos*	0.82	10.1,10.1	Nunney and Elam, 1994
Old World rabbit, *Oryctolagus cuniculus*	0.56	1.8, 1.8	Nunney and Elam, 1994
Grey squirrel, *Sciurus carolinensis*	0.57	1.79,2.44	Nunney and Elam, 1994
Banner-tailed kangaroo rat, *Dipodomys spectabilis*	0.56	1.7,1.7	Nunney and Elam, 1994
Wild horse, *Equus caballus*	0.82	7.23, 7.86	Nunney and Elam, 1994
Moose, *Alces alces*	0.84	4.8,4.8	Nunney and Elam, 1994
American bison, *Bison bison*	0.72	7.0, 7.0	Nunney and Elam, 1994

Estimates of N_e/N for *C. russula*, *H. sapiens*, and *P. cynocephalus* were obtained using the Hill (1972, 1979) equation for age-structured populations. The remaining estimates were obtained using a simplified version of the Hill equation based on the assumption of age-independent survival and fertility (Nunney, 1993; Nunney anc Elam, 1994). T_m and T_f are the mean generation times (in years) for males and females, respectively.

are likely highest in lineages characterized by stochastic variation in population numbers.

Geographic Patterning of Genetic and Phenotypic Variation

Under equilibrium conditions in an island model of population structure, and assuming that the rate of mutation is negligibly small relative to the rate of migration, Wright (1969: 291) demonstrated that $F_{ST} = (1 + 4\, N_e\, m)^{-1}$ (where F_{ST} is the standardized variance in allelic frequencies and $N_e\, m$ is the migration rate scaled by local effective size). Kimura and Weiss (1964) showed that, as a general rule of thumb, the homogenizing effect of gene flow will be sufficient to counteract stochastic divergence via drift when $N_e\, m > 4$. In contrast, the diversifying effect of drift will be sufficient to overpower gene flow when $N_e\, m \ll 1$. Results of our analysis reported above indicate that, in the absence of outside emigration, the Pune study population of *C. sphinx* would be characterized by an instantaneous effective size of 108.2 (N_e/N = 0.42, average N = 257.5 adults; Storz *et al.*, 2001c). Assuming that this estimate is generally applicable to local populations of *C. sphinx* across the region surveyed, a per generation migration rate of > 0.037 would be required to meet the criterion that $N_e\, m > 4$.

To assess the scale and magnitude of spatial differentiation in *C. sphinx*, a total of 251 adults were sampled for a joint analysis of genetic and morphometric variation. Bats were sampled from eight localities along a latitudinal transect that spanned a linear distance of 1080 km across peninsular India (from 18° N to 9° N; Figure 3). Morphometric variation of *C. sphinx* was assessed by examining seven external characters that jointly summarize overall body dimensions and wing area (Storz, 2002; Storz *et al.*, 2001a). Principal components analysis on the variance-covariance matrix of the seven morphometric traits was used to extract a multivariate index of overall body size. Genetic analysis was based on a total of six polymorphic microsatellite loci and partitioning of genetic variance within and among populations was assessed using Weir and Cockerham's (1984) estimators of *F*-statistics.

Inferring the Relative Importance of Drift and Gene Flow in Causing Genetic Differentiation

The geographic patterning of microsatellite variation revealed a relatively low degree of genetic subdivision across peninsular India (F_{ST} =0.030, 95% CI= 0.013-0.045). However, simple summary statistics provide no insight into the relative importance of drift and gene flow in causing the observed level of genetic differentiation. Two separate criteria can be used to determine whether a particular population has attained migration-drift equilibrium: (1) a significant association between pairwise F_{ST} and distance, and/or (2) a scatterplot of pairwise F_{ST} versus distance that reveals a positive and monotonic relationship over the full range of distance values (Hutchison and Templeton, 1999).
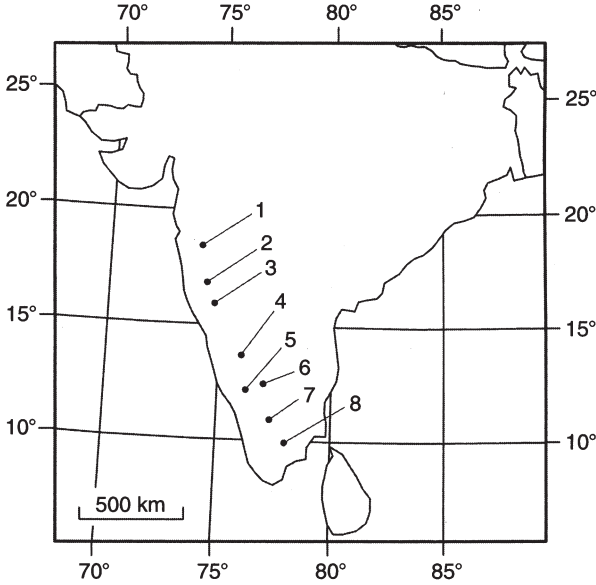
Figure 3. Map of peninsular India showing localities where *Cynopterus sphinx* was sampled for the analysis of morphometric and genetic variation. Names of sampling localities, geographic coordinates, and elevation (recorded to the nearest 10 m) are as follows: (1) Pune, Maharashtra (18°32′ N 73°51′ E, 600 m), (2) Kolhapur, Maharashtra (16°42′ N 74°13′ E, 560 m), (3) Belgaum, Kamataka (15°54′ N 74°36′ E, 900 m), (4) Shimoga, Karnataka (13°56′ N 75°35′ E, 650 m), (5) Thithimathi, Kamataka (12°05′ N 76°00′ E, 860 m), (6) Mysore, Karnataka (12°18′ N 76°37′ E, 780 m), (7) Metupalayam, Tamil Nadu (11°18′ N 76°59′ E, 450 m), and (8) Othakadai, Tamil Nadu (9°56′ N 78°07′ E, 150 m).

Pairwise estimates of F_{ST} exhibited a monotonic increase as a positive function of separation distance (Figure 4). Matrix randomization tests revealed a statistically significant relationship between arcsin $\sqrt{F_{ST}}$ and ln-distance ($r = 0.533$, $P = 0.008$). Absolute standardized residuals from a regression of arcsin $\sqrt{F_{ST}}$ against ln-distance also exhibited a significant positive correlation with ln-distance ($r = 0.401$, $P = 0.041$). Thus, the spatial patterning of pair-wise F_{ST} clearly indicates that *C. sphinx* has attained migration-drift equilibrium under extremely high levels of gene flow across peninsular India. Moreover, the microsatellite data revealed no evidence of major phyletic breaks across the region surveyed (Figure 5). Having verified migration-drift equilibrium among the sampled populations, the overall F_{ST} value translates into an estimate of $N_e m = 8.08$ using Weight's (1969) infinite alleles/island model approximation. As might be expected for a species with such strong dispersal capabilities, this $N_e m$ estimate far exceeds the level required to counteract stochastic divergence via drift.

Genetic evidence suggests that *C. sphinx* has undergone a historical population contraction in the Indian subcontinent, possibly as a result of climatically induced range shifts during the late Quaternary (Storz and Beaumont,
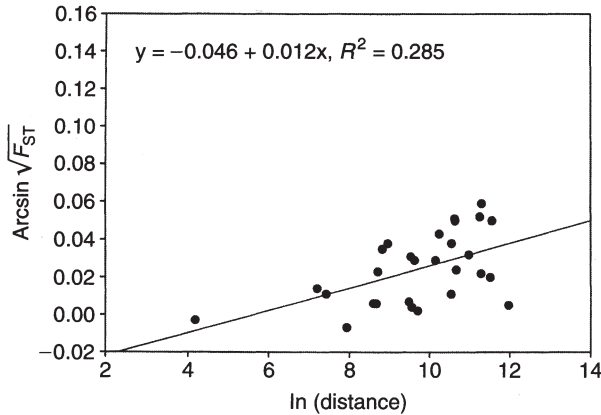
Figure 4. Least-squares linear regression of arcsin $\sqrt{F_{ST}}$ against ln-distance for each pair-wise combination of populations. Statistical significance of regression coefficients was assessed using a matrix randomization test.
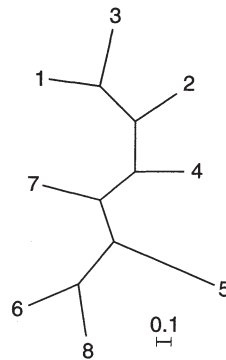

Figure 5. Neighbor-joining phenogram of *C. sphinx* populations in peninsular India, based on a matrix of the co-ancestry distance of Reynolds *et al.* (1983; equation 5.12 in Weir, 1996). The consensus phenogram was based on 1000 bootstrap replicates of the microsatellite data set. Numbers refer to populations listed in the legend for Figure 3.

2002). Thus, we might expect *C. sphinx* to be characterized by a nonequilibrium mode of population structure that reflects the predominant role of drift relative to gene flow. This mode of population structure would be implicated by a random association between pairwise F_{ST} and separation distance in conjunction with a wide degree of scatter between plotted points (Hutchison and Templeton, 1999). However, since the observed pattern of microsatellite variation in *C. sphinx* is clearly consistent with an isolation-by-distance relationship, levels of gene flow across peninsular India must have been high enough to maintain (or re-establish) migration-drift equilibrium following the historical reduction in effective population size.

   Since F_{ST} is based on the infinite alleles model of mutation (Kimura and Crow, 1964), its suitability for the analysis of microsatellite variation de-

pends on the spatial and/or temporal scale of divergence under consideration. Slatkin (1991, 1993) derived expressions for inbreeding coefficients in terms of allelic genealogies and demonstrated that F_{ST} measures the difference in within- and between-population coalescence times scaled by the average coalescence time. As a measure of genetic divergence, F_{ST} is therefore independent of mutation rate (μ), provided that the average coalescence time is less than $1/\mu$. Simulation results of Slatkin (1993) indicate that high mutation rates characteristic of microsatellite loci could potentially mask a pattern of isolation by distance. However, for the spatial scale considered in this study, rates of migration are so high relative to possible rates of mutation that Weir and Cockerham's (1984) unbiased estimator of F_{ST} (= θ) can be expected to provide a more accurate measure of genetic differentiation than statistics based on the stepwise mutation model (Gaggiotti *et al.*, 1999; Slatkin, 1995).

Since simple-sequence repeats are largely restricted to noncoding regions of the genome, microsatellite variation is generally considered to be selectively neutral (Schlötterer and Wiehe, 1999). The validity of this assumption was evaluated for the markers used in this study by comparing observed F_{ST} values with a null distribution of values generated by a coalescent-based simulation model. Specifically, the model of Beaumont and Nichols (1996) was used to generate the expected neutral distribution of F_{ST} as a function of heterozygosity. Coalescent simulations were performed using a symmetrical 100-island model of population structure, with sample sizes of 30 diploid individuals (= median of actual sample sizes). Two separate sets of simulations were performed in which mutational dynamics conformed to either the infinite alleles model or the stepwise mutation model. In order to generate a wide range of heterozygosity values, simulations were based on two different mutation rates ($N_e\mu$ = 0.1 and 1.0, where $N_e\mu$ is the mutation rate scaled to effective population size). Results of the simulations revealed no evidence for departures from neutral expectations at any locus, regardless of the underlying mutation model ($P > 0.05$ for every locus × model combination). When single-locus F_{ST} values were plotted as a function of heterozygosity, observed points were well within the 0.025 and 0.975 quantiles of the expected neutral distribution.

Testing for Evidence of Spatially Varying Selection as a Cause of Phenotypic Differentiation

Clinal variation in quantitative traits is often attributed to the effects of spatially varying selection across an environmental gradient. However, identical patterns can be produced by the interplay between purely stochastic processes. For example, clinal variation in allelic frequencies at genes underlying a particular trait can result from gene flow between partially isolated populations that have diverged via drift, or admixture between two or more genetically differentiated founding populations. One means of inferring the role of selection in the maintenance of clinal variation is to compare relative levels of between-population divergence in quantitative traits and neutral DNA

markers. Such comparisons can be used to test whether the observed level of trait divergence exceeds neutral expectations (Lande, 1992; Lynch, 1994; Rogers and Harpending, 1983; Whitlock, 1999). When a species is distributed across an environmental selection gradient, a joint analysis of phenotypic divergence and isolation by distance for neutral DNA markers can elucidate the spatial scale at which adaptation to local environmental conditions can evolve in response to spatially varying selection (Slatkin, 1973).

If the geographic patterning of additive genetic variance underlying a particular trait is exclusively attributable to migration-drift equilibrium (and if there are no departures from allelic or genotypic equilibria within populations), variance components can be defined as $\sigma_b^2 = 2 F_{ST} \sigma_o^2$, $\sigma_w^2 = (1 - F_{ST}) \sigma_o^2$, and $\sigma_t^2 = (1 + F_{ST}) \sigma_o^2$, where σ_w^2, σ_b^2, and σ_t^2 represent the within-population, between-population, and total genetic variances in trait values, respectively, and σ_o^2 represents the total variance in the trait under panmixia (Lande, 1992; Rogers and Harpending, 1983; Wright, 1951). Thus, a dimensionless measure of differentiation for quantitative traits, analogous to Weight's (1951) F_{ST}, can be defined as

$$Q_{ST} = \sigma_b^2 / (\sigma_b^2 + 2 \sigma_w^2). \tag{4}$$

The partitioning of phenotypic variance within and between populations of *C. sphinx* was assessed using a two-way ANOVA on PC1 scores, with sex included as a fixed-effects factor. Using the method-of-moments approach outlined in Storz (2002), variance components were estimated by equating observed mean squares to their expectations. Accordingly, Q_{ST} for body size of *C. sphinx* was calculated for each pairwise combination of populations using moment-based estimates of the within- and between-population variance components (Var(w) and Var(b), respectively) and within-population phenotypic variance (×0.5) was used as a proxy for additive genetic variance (equivalent to assuming that body size is characterized by a narrow-sense heritability of $h^2 = \frac{1}{2}$).

In peninsular India, the geographic pattern of variation in external morphology of *C. sphinx* conforms to Bergmann's rule, as indicated by a steep, monotonic cline of increasing body size from South to North (Figure 6; Storz *et al.*, 2001a). If clinal size variation of *C. sphinx* is simply attributable to isolation by distance, the positive association between Q_{ST} and separation distance should disappear when the effects of neutral genetic divergence (as measured by F_{ST} for microsatellites) are held constant. By contrast, the null hypothesis of isolation by distance would be rejected if the increase in pairwise Q_{ST} as a function of geographic/environmental distance remained statistically significant after controlling for pairwise F_{ST}. A significant partial regression of pairwise Q_{ST} on distance would indicate that migration-drift equilibrium is not a sufficient explanation for the latitudinal pattern of clinal size variation in *C. sphinx*.
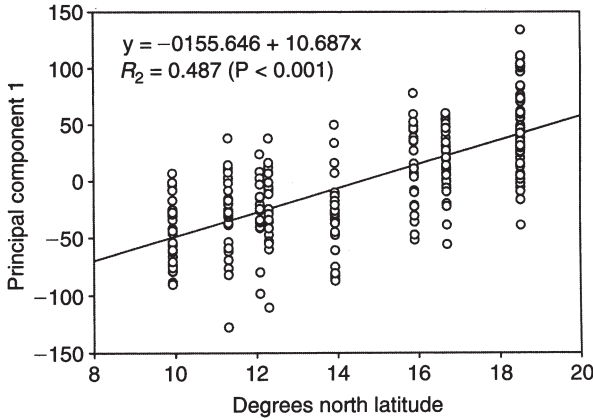
Figure 6. Least-squares linear regression of body size (as indexed by PC1 scores) against latitude for *Cynopterus sphinx* ($n = 251$) sampled from peninsular India.

The association between body-size variation and geographic/environmental distance was tested using pairwise and partial Mantel tests (Manly, 1997). Using a stepwise multiple regression procedure, a matrix of arcsin $\sqrt{Q_{ST}}$ (**Q**) was related to a matrix of arcsin $\sqrt{F_{ST}}$ (**F**) and a matrix of pairwise measures of geographic or environmental distance (**D**). For the three respective matrices, let q_{ij}, f_{ij}, and d_{ij} denote distances between localities i and j. The following model was then evaluated:

$$q_{ij} = \beta_0 + \beta_1 f_{ij} + \beta_2 d_{ij} + \varepsilon_{ij} \tag{5}$$

where β_2 measures the association between q_{ij} and d_{ij} while controlling for the effects of f_{ij}, and ε_{ij} represents an independent error term. Statistical significance of the association between the dependent variable matrix **Q** and the two independent variable matrices (**F** and **D**) was assessed by means of a randomization test (Manly, 1997). In addition to the tests based on linear measures of geographic distance, partial Mantel tests were performed using multivariate measures of pairwise environmental distance (see Storz, 2002). In all pairwise and partial Mantel tests, the matrix element representing the Thithimathi-Mysore comparison was excluded since these two localities were closely situated at the same latitude.

Across the entire transect, the average pairwise estimate of Q_{ST} was 13.5-fold larger than that of F_{ST} (0.323 *vs.* 0.024). In contrast to the levels of population subdivision observed at microsatellite loci, pairwise Q_{ST} exhibited a remarkably steep increase as a positive function of distance (Figure 7). Pairwise Mantel tests revealed highly significant correlations between Q_{ST} and the following variables: F_{ST}, geographic distance, and an environmental distance metric based on a temperature/equability vector (PC1-T; Table 2). The correlation between Q_{ST} and F_{ST} is primarily attributable to the large number of cases where both measures were close to zero. Regression coefficients
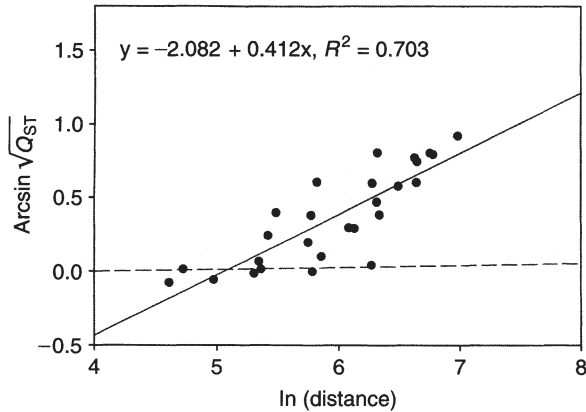
Figure 7. Least-squares linear regression of arcsin $\sqrt{Q_{ST}}$ against ln-distance for each pairwise combination of populations. The dashed line denotes the linear regression line for arcsin $\sqrt{F_{ST}}$ versus distance (note the difference in scale of the y-axis compared with Figure 4). Statistical significance of the regression coefficient was assessed using a matrix randomization test.

for the matrices of geographic and environmental distance remained highly significant in the partial Mantel tests (Table 2). In other words, the increase in Q_{ST} as a positive function of geographic and environmental distance remained statistically significant even when the effects of neutral divergence (F_{ST}) were partialed out. Regression coefficients for matrices of geographic and environmental distance also remained significant when randomizations were restricted to spatially defined groups of populations along the transect (Storz, 2002). Thus, we can be confident that the regression coefficients reflect real associations and cannot be explained as artifacts of spatial autocorrelation (Oden and Sokal, 1992; Raufaste and Rousset, 2001).

In conclusion, results of the partial Mantel tests confirmed that migration-drift equilibrium is not a sufficient explanation for the latitudinal pattern of clinal size variation in *C. sphinx*. Between-population divergence in body size increased with environmental distance across a South-to-North gradient of decreasing temperature, decreasing relative humidity, and increasing seasonality (Storz *et al.* 2001a, Storz, 2002). The geographic patterning of pairwise Q_{ST} is most likely attributable to spatially varying selection and/or the direct influence of latitudinally ordered environmental effects.

Inferences about the adaptive basis of clinal variation are strengthened when the environmental component of phenotypic variation can be identified and statistically removed (Coyne and Beecham, 1987; Huey *et al.*, 2000; Long and Singh, 1995; Mousseau and Roff, 1989, 1995). This can be accomplished by comparing different populations using common-garden or reciprocal transplant experiments (Mousseau, 1999). Unfortunately, this approach is not readily tractable for the study of bat populations. To examine the effects

Table 2. Results of Pairwise (A) and Partial (B) Mantel Tests of Causal Hypotheses Regarding the Pattern of Clinal Size Variation in C. *sphinx*

	Independent variable matrices			
	Arcsin $\sqrt{F_{ST}}$	ln-distance	PC1-T distance	PC1-R distance
(A) Pairwise MCTs				
arcsin $\sqrt{Q_{ST}}$	**0.634 (P = 0.0003)**	**0.838 (P = 0.0001)**	**0.463 (P = 0.0007)**	-0.082 (P= 0.6970)
(B) Partial MCTs				
arcsin $\sqrt{Q_{ST}}$	**0.303 (P = 0.0020)**	**0.694 (P = 0.0001)**	—	—
arcsin $\sqrt{Q_{ST}}$	**0.743 (P = 0.0001)**	—	**0.599 (P = 0.0004)**	—

Partial Mantel tests were performed in a stepwise regression procedure for variables that showed a significant degree of association in pairwise tests. A matrix of arcsin $\sqrt{F_{ST}}$ was included as an independent variable in each of the partial regression analyses to control for the effects of neutral genetic divergence. Tests were performed on independent variable matrices of pairwise ln-distance and two separate pairwise measures of environmental distance (indexed by PC1-T and PC1-R vectors). PC1-T and PC1-R are multivariate axes that summarize latitudinal variation in temperature and precipitation, respectively. Standardized regression coefficients and associated P values that remained statistically significant after Bonferroni correction are in bold. P values for one-sided tests are expressed as the proportion of 10,000 randomizations that yielded values greater than or equal to observed t values. Since the pairwise MCT revealed no significant matrix correlation between arcsin $\sqrt{Q_{ST}}$ and PC1-R distance, the latter variable was not included in the partial MCT with the PC1-T distance matrix.

of different assumptions about the genetic basis of body size variation in *C. sphinx*, Q_{ST} was recalculated over a range of values for Var(w) (assuming h^2 in the range 0.50–0.85) and Var(b) (assuming that 50–85% of the between-population variance was attributable to a nonheritable environmental component). Results of this exercise indicate that over the full range of biologically plausible values for Var(w) and Var(b), the linear regression slope for arcsin $\sqrt{Q_{ST}}$ versus ln-distance remained significantly steeper than that for arcsin $\sqrt{F_{ST}}$ versus ln-distance. When it was assumed that $h^2 = 0.85$ and that 85% of the between-population variance in body size was attributable to environmental effects (%Var(b) genetic =15), partial Mantel tests between the recalculated Q_{ST} matrix and each of the geographic and PC1-T distance matrices remained statistically significant ($P < 0.05$). Thus, the environmental component of the between-population variance in body size would have to be extraordinarily large to accept the null hypothesis of neutral phenotypic divergence.

In studies of geographic variation, inferences about the interplay between different evolutionary forces are greatly enhanced when patterns of genetically based trait variation are considered in conjunction with estimates of neutral genetic divergence (*e.g.,* Prout and Barker, 1993; Spitze, 1993). As demonstrated in our analysis of clinal size variation in *C. sphinx*, a matrix of pairwise estimates of neutral genetic divergence can be used as an independent variable in a partial Mantel test when the observed pattern of phenotypic divergence is tested against causal hypotheses. The chief merit of the approach based on Q_{ST} versus F_{ST} contrasts is that results can be interpreted within the framework of the neutral theory of phenotypic evolution (reviewed by Lynch, 1994).

Potential Causes of Latitudinal Variation in Phenotypic Optima

If the observed pattern of clinal size variation in *C. sphinx* has an adaptive genetic basis, what are the underlying causes of spatially varying selection? In pteropodid bats, basal metabolic rate is highly size-dependent and medium-sized frugivores such as *C. sphinx* are typically characterized by precise regulation of body temperature (McNab, 1989; McNab and Bonaccorso, 1995). Because the energetics of temperature regulation have important consequences for fecundity, gestation period, and rates of postnatal growth in bats (McNab, 1982), the ecologically optimal body size of nonmigratory species may be expected to vary across broad-scale climatic gradients. Although the underlying causes remain to be elucidated, it seems clear that spatially varying selection has played an important role in shaping latitudinal size variation in *C. sphinx.*

Geographic Variation, Clines, and Subspecies

There is considerable uncertainty surrounding the taxonomic status of the many named forms of *C. sphinx* in the Indomalayan Region (reviewed by Storz and Kunz, 1999). Larger specimens from northern India were originally referred to *C. sphinx gangeticus*, while generally smaller specimens from the

south were referred to *C. sphinx sphinx* (Andersen, 1912; Hill, 1983). Results of the analysis reported here indicate that the nominal subspecies *C. s. gangeticus* and *C. s. sphinx* represent two ends of a continuous spectrum of clinal size variation. The combined analysis of genetic and phenotypic variation indicates that subspecies boundaries in peninsular India represent arbitrary subdivisions of a continuous gradation of morphological characters. Imposing a formal nomenclature on this gradation of form does nothing to elucidate the underlying causes of geographic variation. It remains to be seen whether other examples of clinal variation in bat populations represent cases of selection-migration equilibrium across an environmental gradient, isolation by distance, or secondary intergradation across a contact zone between phylogenetically distinct forms.

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