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Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analysis of clinal variation

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Abstract

Clinal variation in quantitative traits is often attributed to the effects of spatially varying selection. However, identical patterns can be produced by the interplay between purely stochastic processes (i.e. drift in combination with spatially restricted gene flow). One means of distinguishing between adaptive and nonadaptive causes of geographical 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 levels of trait divergence attributable to additive genetic effects (as measured by Q_{ST}) exceed null expectations based on the level of divergence at neutral marker loci (as measured by F_{ST}). The purpose of this study was to use an approach based on 'Q_{ST} vs. F_{ST}' contrasts to test for evidence of diversifying selection on body size of an Indian fruit bat, *Cynopterus sphinx* (Chiroptera: Pteropodidae). Specifically, relative levels of between-population divergence in body size and microsatellite DNA markers were compared to assess whether the observed pattern of clinal size variation could be explained by a neutral model of isolation by distance. Q_{ST} for body size was calculated using unbiased estimators of within- and between-population variance of principal component scores. The association between body size variation and geographical/environmental distance was tested using pairwise and partial matrix correspondence tests (MCTs). Independent variables (representing causal hypotheses) were constructed as between-locality distance matrices. The effects of neutral genetic divergence were assessed by including a matrix of pairwise F_{ST} as an independent variable. Partial MCTs revealed highly significant associations between phenotypic divergence (Q_{ST}) and both geographical and environmental distance, even when the effects of neutral genetic divergence (F_{ST}) were partialled out. Results of the tests confirmed that migration–drift equilibrium is not a sufficient explanation for the latitudinal pattern of clinal size variation in *C. sphinx*. The geographical patterning of pairwise Q_{ST} is most likely attributable to spatially varying selection and/or the direct influence of latitudinally ordered environmental effects.

Keywords: bats, Bergmann's rule, *Cynopterus sphinx*, isolation by distance, microsatellite DNA, phenotypic divergence, Q_{ST}

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Introduction

Clinal variation in genetically based traits can provide compelling evidence for spatially varying selection across an environmental gradient (Haldane 1948; Slatkin 1973, 1978; Endler 1977). Clines are of particular interest in highly vagile species because their persistence requires recurrent selection to counterbalance the homogenizing effect of gene flow. However, there are also alternative explanations for the stable maintenance of clinal variation that do not invoke spatially varying selection. Clinal variation in allelic frequencies at genes underlying a particular trait can result from gene flow between partially isolated populations that have diverged in genetic composition via drift, or admixture between two or more genetically differentiated founding populations. As stated by Gould & Johnston (1972: p. 459), '… [clines] can be just as well explained by a nonselective isolation-by-distance model as by an appeal to selection by correlated environmental factors.' The

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relative importance of adaptive and nonadaptive causes of clinal variation is an issue of central importance to our understanding of local adaptation and the determinants of geographical variation (Mayr 1963; Felsenstein 1976; Endler 1977; García-Ramos & Kirkpatrick 1997).

In some cases, an inductive approach can be used to infer the causes of clinal variation in ecologically important traits. Because stochastic processes such as drift and gene flow are not expected to produce systematic patterns of variation in allelic frequencies, parallel clines in geographically isolated populations of the same species strongly implicate an adaptive basis for geographical differentiation. For example, parallel clines in body size of *Drosophila subobscura* on different continents implicate spatially varying selection as the driving force of phenotypic divergence across latitudinal gradients (Huey *et al*. 2000). A more general approach to infer the role of selection in maintaining clinal variation is to compare relative levels of between-population divergence in quantitative traits and neutral DNA markers. When testing for the effects of spatially varying selection, the null hypothesis is that the level of trait divergence attributable to additive genetic effects (as measured by Q_{ST}) does not exceed the level of divergence at neutral loci (as measured by F_{ST}; Wright 1943, 1951; Rogers & Harpending 1983; Zeng & Cockerham 1991; Prout & Barker 1993; Spitze 1993).

Phenotypic divergence and isolation by distance

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 conditions can evolve in response to diversifying selection. Consider the case of a species with strong dispersal capabilities (high gene flow) that is distributed across a continuous climatic gradient where environmental similarity is a linear function of spatial proximity. If selection on a particular trait is mediated by spatially varying environmental factors, the disparity in trait optima between populations increases as a function of distance. In accordance with the intraspecific interpretation of Bergmann's rule (Mayr 1963; James 1970), such a pattern might be expected when populations of a homeothermic animal species are sampled across a latitudinal gradient in temperature, humidity, rainfall, or other factors that influence local optima for body size.

If the observed cline in trait values is solely attributable to isolation by distance, Q_{ST} for the trait and F_{ST} for neutral markers should exhibit concordant patterns of increase with geographical distance. Conversely, if the cline in trait values reflects adaptation to spatially varying environmental conditions, Q_{ST} for the trait should exceed F_{ST} for neutral markers. The role of selection in maintaining clinal variation is most clearly revealed when a species has not attained migration–drift equilibrium under high levels of gene flow (such that neutral loci do not conform to an isolation-by-distance relationship). Under such circumstances, Q_{ST} is expected to increase as a positive function of distance while F_{ST} should exhibit no significant correlation with distance at any spatial scale.

The purpose of this study was to use an approach based on 'Q_{ST} vs. F_{ST}' contrasts to test for evidence of diversifying selection on body size of an Indian fruit bat, *Cynopterus sphinx* (Chiroptera: Pteropodidae). Specifically, relative levels of between-population divergence in body size and microsatellite DNA markers were compared to assess whether the observed pattern of clinal size variation can be explained by a neutral model of isolation by distance.

Materials and methods

Study species, study area, and sampling design

Cynopterus sphinx is a medium-sized fruit bat with an average wingspan of ~380 mm (Bates & Harrison 1997; Storz & Kunz 1999). It is one of the most widely distributed and abundant fruit bats in the Indo-Malayan Region, and is not known to undergo seasonal migrations (Corbet & Hill 1992; Storz & Kunz 1999). In peninsular India, the geographical 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 (Storz *et al*. 2001a). In the present study, a total of 251 adult *C. sphinx* (95 males and 156 females) were sampled for a joint analysis of morphometric and genetic variation. Bats were sampled from eight localities along a latitudinal transect that spanned a linear distance of 1080 km across peninsular India, from 18°32′ N, 73°51′ E (Pune) to 9°56′ N, 78°07′ E (Othakadai; Fig. 1). All available evidence indicates that *C. sphinx* is continuously distributed across the region surveyed in this study (Corbet & Hill 1992; Bates & Harrison 1997; Storz & Kunz 1999). The latitudinal transect was orientated along the eastern flanks of the Western Ghats, a mountain range that parallels the western coast of the subcontinent. The crestline of the Western Ghats averages 900–1500 m in elevation and intercepts the southwest monsoon, thereby creating a rain shadow across the semiarid plains to the east. Sampling localities were situated in semiarid tropical thorn-scrub/secondary forest habitat (Mani 1974b; Subramanyam & Nayar 1974) at elevations ranging from 30 to 900 m. The latitudinal transect spanned a north-to-south gradient of increasing temperature and relative humidity (Mani 1974a; Ramdas 1974; Storz *et al*. 2001a). The annual range of ambient temperature also varies with latitude in peninsular India, decreasing from the more seasonal Deccan Plateau in the north to the more equable climate of the Tamilnad Plains in the south.

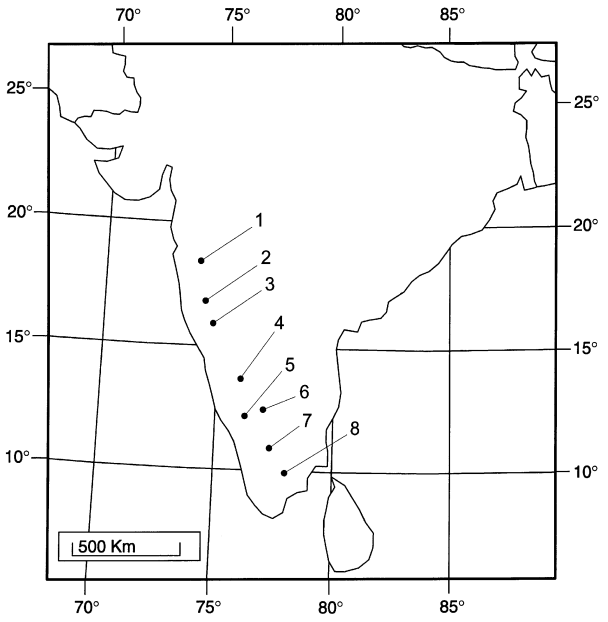

Fig. 1 Map of peninsular India showing localities where *Cynopterus sphinx* was sampled for the joint analysis of morphometric and genetic variation. Names of sampling localities, geographical coordinates, and elevation (recorded to the nearest 10 m) are as follows: 1, Pune, Maharashtra (18°32′ N 73°51′ E, 600 m); 2, Kolhapur, Maharashtra (16°42′ N 74°13′ E, 560 m); 3, Belgaum, Karnataka (15°54′ N 74°36′ E, 900 m); 4, Shimoga, Karnataka (13°56′ N 75°35′ E, 650 m); 5, Thithimathi, Karnataka (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).

Bats were trapped at foraging and roosting sites (Storz *et al*. 2000a,b 2001a) and wing-membrane biopsies were taken as a source of DNA for the genetic analysis (see Storz *et al*. 2001b,c). All bats were sampled during an 8-week period during the dry-season breeding period (4 March to 2 May 1998). Each individual was classified as juvenile or adult based on the degree of fusion of the metacarpal–phalangeal epiphyses (Anthony 1988). Only adults were used in the subsequent analysis of morphometric and genetic variation, and bats were released at the site of capture after processing.

Analysis of morphometric variation

Morphometric variation of *C. sphinx* was assessed by examining eight external characters that jointly summarize overall body dimensions and wing area (Storz *et al*. 2001a): length of tibia, length of forearm, length of metacarpal of digits 2–5, proximal phalanx of digit 3, and body mass. Univariate normality and equality of error variances were confirmed for $\log_{10}$-transformed values of each trait.

In bats, a multivariate axis that summarizes body and wing dimensions can provide a functionally relevant measure of overall structural size (Storz *et al*. 2001a). Accordingly, principal components analysis was performed on the variance–covariance matrix of seven $\log_{10}$-transformed morphometric variables (all characters except body mass) to extract a multivariate index of overall body size. If the first axis of a principle components analysis (PC1) provides an accurate and functionally meaningful representation of overall structural size, PC1 scores and body mass should covary in a positive, linear fashion (Rising & Somers 1989). This relationship was tested by means of bivariate correlation analysis. Sexual dimorphism and geographical variation in sexual dimorphism were examined by performing a mixed model two-way analysis of variance (ANOVA) on PC1 scores with sex and geographical locality included as fixed-effects and random-effects factors, respectively. Additionally, sex differences in patterns of clinal size variation were examined by testing for differences in slopes from a regression of PC1 scores on latitude (Zar 1999; pp. 360–364).

If the geographical 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 $\sigma_b^2 = 2F_{ST}\,\sigma_0^2$, $\sigma_w^2 = (1 - F_{ST})\,\sigma_0^2$, and $\sigma_t^2 = (1 + F_{ST})\,\sigma_0^2$ where σ_w^2, σ_b^2, and σ_t^2 represent the within-population, between-population, and total genetic variances in the trait, respectively, and σ_0^2 represents the total variance in the trait under panmixia (Wright 1943, 1951; Rogers & Harpending 1983; Lande 1992; Spitze 1993). Thus, a dimensionless measure of differentiation for quantitative traits, analogous to Wright's (1951) F_{ST}, can be defined as

$$Q_{ST} = \frac{\sigma_b^2}{\sigma_b^2 + 2\sigma_w^2} \quad (1)$$

(Wright 1951; Spitze 1993). The partitioning of phenotypic variance within and between populations of *C. sphinx* was assessed using a two-way ANOVA on PC1 scores, as above, with sex included as a fixed-effects factor. Variance components were estimated by equating observed mean squares (*MS*) to their expectations (Spitze 1993; Lynch & Walsh 1998). MS_{within} is an unbiased estimate of the within-population variance (σ_w^2) and $MS_{between}$ is an unbiased estimate of the between-population variance ($\sigma_w^2 + n_0\,\sigma_b^2$), where n_0 is the average sample size and σ_b^2 is the added variance component attributable to differences between populations (Sokal & Rohlf 1995; pp. 208–217). For each comparison, the average sample size n_0 was calculated as

$$n_0 = \frac{1}{a-1}\left(\sum_a^a n_i - \frac{\sum_a^a n_i^2}{\sum_a^a n_i}\right) \quad (2)$$

where a = number of populations compared and n = number of individuals in the ith population sample. The

added component of variance between populations was estimated as

$$\mathrm{Var}(b) = (MS_{\mathrm{between}} - MS_{\mathrm{within}})/n_0 \quad (3)$$

Using estimates of the within- and between-population variance components [Var(w) and Var(b), respectively], Q_{ST} for body size of *C. sphinx* was calculated for each pairwise combination of populations using eqn 1. Standard errors for estimates of variance components were obtained using the delta method (Kendall & Stuart 1977; Lynch & Walsh 1998).

Q_{ST} can be interpreted as an F_{ST}-analogue for quantitative traits provided that within- and between-population variance in trait values is exclusively attributable to additive genetic effects (Wright 1943; Rogers & Harpending 1983). Otherwise, Q_{ST} estimates may be biased if within- and between-population components of environmental variance are not proportional. Since data in the present study are purely phenotypic, estimation of within-population variance requires an assumption about heritability of body size. Following Merilä (1997), Q_{ST} for body size of *C. sphinx* was calculated by substituting the observed phenotypic variance for 2Var(w). This is equivalent to assuming that body size (as indexed by PC1) has a narrow-sense heritability (h^2) of 0.5. Empirical estimates of h^2 for mammalian body size suggest that this is a reasonable assumption (Falconer & Mackay 1996). As will be demonstrated, the environmental component of the between-population variance in body size would have to be extremely large (and h^2 would have to be extremely high) to accept the null hypothesis of neutral phenotypic divergence.

Analysis of microsatellite DNA variation

Genomic DNA was isolated from tissue samples of *C. sphinx* using QIAamp extraction columns (Qiagen Inc., Valencia, CA). Genetic analysis was based on a total of six polymorphic microsatellite loci: two di-, two tri-, and two tetranucleotide repeats. Primer sequences and polymerase chain reaction protocols were reported previously (Storz 2000; Storz *et al*. 2001b,c). Allele sizes were quantified using a 377 ABI Prism automated sequencer and analysed using genescan software (PE Applied Biosystems). Sequencing of alleles confirmed that length polymorphism at each locus was attributable to variation in the copy-number of a single repeat motif. Nei's (1987) average gene diversity (H) was computed for each population, and arcsine-transformed values were used to test for a correlation with latitude. Partitioning of genetic variance within and among populations was assessed using Weir & Cockerham's (1984) estimators of F-statistics: $f(= F_{IS})$, $F(= F_{IT})$, and $\theta(= F_{ST})$. Ninety five per cent confidence intervals were obtained by bootstrapping over loci. Null hypotheses of Hardy–Weinberg genotypic proportions ($F_{IS} = 0$ and $F_{IT} = 0$) were tested using a randomization procedure. Null distributions were generated from 10 000 randomizations of alleles among individuals within populations (for F_{IS}) and among individuals sampled across the total array of populations (for F_{IT}). Since F_{ST} is based on the infinite alleles model of mutation (Kimura & Crow 1964), its suitability for the analysis of microsatellite variation depends 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. For comparative purposes, genetic divergence was also assessed using R_{ST} (based on the stepwise mutation model; Ohta & Kimura 1973; Valdes *et al*. 1993; Slatkin 1995). After standardizing allele lengths (zero mean, unit variance), Goodman's (1997) ρ was used as an estimator of R_{ST}. The programs fstat version 2.9.1 (updated from Goudet 1995) and R_{ST} calc (Goodman 1997) were used for all calculations.

Since simple-sequence repeats are largely restricted to noncoding regions of the genome, microsatellite variation is generally considered to be selectively neutral (Schlötterer & Wiehe 1999). The validity of this assumption was evaluated for the markers used in this study by comparing observed F_{ST} values to a null distribution of values generated by a coalescent-based simulation model. Specifically, the model of Beaumont & Nichols (1996) was used to generate the expected neutral distribution of F_{ST} as a function of single-locus 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. 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).

Testing the neutral model of isolation by distance

Measures of genetic differentiation (hereafter, 'F_{ST}') were calculated for all pairwise combinations of populations, as were straight-line geographical distances. To test for isolation by distance, pairwise F_{ST} values were arcsine-transformed and regressed against ln-transformed measures of separation distance. Since the nonindependence of

pairwise comparisons precludes the use of parametric tests based on the *t*- or *F*-distributions, statistical significance of regression coefficients was assessed by means of a matrix randomization test (Manly 1997). A computer program was used to create a null distribution of regression coefficients by iteratively randomizing the order of elements in the dependent variable matrix. Probability values for the null hypothesis of no association were then expressed as the proportion of 10 000 randomizations that yielded *t* values greater than or equal to the observed value. To test for an increase in residual variance of pairwise F_{ST} as a positive function of distance, residuals from the linear regression of arcsin $\sqrt{F_{ST}}$ on ln-distance were regressed against ln-distance. Again, statistical significance was assessed using matrix randomization.

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 geographical/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*.

The association between body size variation and geographical/environmental distance was tested using pairwise and partial matrix correspondence tests (MCTs; Thorpe 1996; Manly 1997; Malhotra & Thorpe 2000). Pairwise MCTs involve comparisons of one dependent and one independent variable matrix, while partial MCTs involve simultaneous tests of multiple independent matrices (also known as 'multiple Mantel tests'; Manly 1997). To test causal hypotheses about clinal size variation in *C. sphinx*, partial MCTs were performed using a stepwise multiple regression procedure for ecogeographic variables that showed a significant degree of association in pairwise tests. For the partial MCTs, 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 geographical or environmental distance (**D**; see below). For the three respective matrices, let q_{ij}, f_{ij}, and d_{ij} denote distances between localities *i* and *j*. The following multiple regression model was then evaluated:

$$q_{ij} = \beta_0 + \beta_1 f_{ij} + \beta_2 d_{ij} + \varepsilon_{ij} \quad (4)$$

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. The 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 geographical distance, MCTs were performed using pairwise measures of environmental distance. The purpose was to examine the relationship between size variation and the following climatological variables: mean annual temperature, mean maximum daily temperature, mean minimum daily temperature, mean annual range in temperature, mean relative humidity, mean annual rainfall, mean maximum rainfall during the wettest month, and mean minimum rainfall during the driest month. Long-term meteorological data were obtained for each sampling locality as described in Storz *et al.* (2001a). To control for multicollinearity, temperature and precipitation variables were reduced to a smaller set of orthogonal vectors by means of principal components analysis on the correlation matrix. Climatic principal components were then used to construct distance matrices for use as independent variables in the partial MCT. Distances for each matrix element were computed as $\sqrt{(X_{ki} - X_{kj})^2}$, where X_{ki} and X_{kj} represent principal component scores on the *k*th axis in the *i*th and *j*th populations. In all pairwise and partial MCTs, the matrix element representing the Thithimathi–Mysore comparison was excluded because these two localities were closely situated at the same latitude (Fig. 1).

A potential problem with partial MCTs arises when two or more explicative factors (e.g. independent matrices of pairwise F_{ST} and pairwise distance) are involved in predicting the elements of a response value (e.g. the matrix of pairwise Q_{ST}). If permutations of the response matrix do not preserve the spatial order of the geographical localities, the effects of spatial autocorrelation will not necessarily be removed by including a matrix of pairwise F_{ST} values in the multiple regression model. In the presence of spatial autocorrelation, the same permutation procedure that is valid for testing the independence of two matrices (the traditional Mantel test) is not necessarily valid when testing for partial effects of a second predictive factor. Consequently, the *P* value derived from the matrix permutations may not be indicative of the true Type 1 error (Oden & Sokal 1992; Raufaste & Rousset 2001). One solution to this problem is to apply a form of restricted randomization that accounts for potential sources of autocorrelation in the data (Sokal *et al.* 1989, 1990; Manly 1997; pp. 188–194). Accordingly, the partial MCTs were performed in such a way that permutations of the response matrix were restricted to spatially defined groups of populations. To account for potential sources of autocorrelation in the data, the following regression model was fitted:

$$q_{ij} = c_{v(i)v(j)} + \beta_1 f_{ij} + \beta_2 d_{ij}, \quad (5)$$

where $c_{v(i)v(j)}$ is a nuisance parameter that takes into account the overall difference between populations in group *i* and populations in group *j*. This model permits an

assessment of the association between pairwise Q_{ST} and geographical or environmental distance after allowing for large-scale differences between spatially defined groups of populations, as well as small-scale differences within groups.

Results

Morphometric variation

The first axis of the principal components analysis (PC1) explained 84.6% of variance in the external morphology of *Cynopterus sphinx* (eigenvalue = 5.92) and was clearly interpretable as an overall size vector as factor loadings for all characters were uniformly high and positive. Product–moment correlations between the original variables and PC1 factor scores were as follows: $\log_{10}$-length of tibia, $r = 0.890$; $\log_{10}$-length of forearm, $r = 0.912$; $\log_{10}$-length of metacarpal of digits 2–5, $r = 0.943$, 0.955, 0.941, and 0.940, respectively; and $\log_{10}$-proximal phalanx of digit 3, $r = 0.853$. Moreover, in a sample of 232 bats (95 males and 137 nonpregnant females), PC1 was strongly and positively correlated with $\log_{10}$-body mass ($r = 0.791$, $P < 0.001$). No statistically significant departure from normality was detected in the total sample of PC1 scores (one sample Kolmogorov–Smirnov test; $Z = 0.759$, $n = 251$, $P = 0.611$). The two-way ANOVA revealed a highly significant degree of heterogeneity among localities ($P < 0.001$), but no significant effects of sex ($P > 0.05$) or sex × locality interaction ($P > 0.05$). Moreover, there was no statistically significant sex difference in the slopes of linear regressions of PC1 on latitude ($t = 1.269$, sum of residual d.f. = 247, $P > 0.05$). Mean PC1 scores exhibited a progressive increase from south to north, indicating a latitudinal cline in overall body size (Fig. 2).

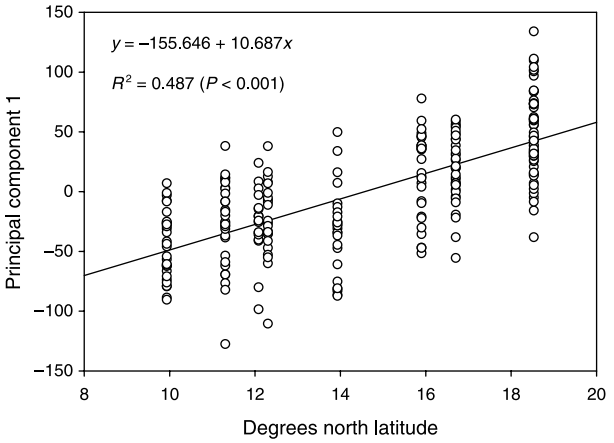

Fig. 2 Least-squares linear regression of body size (as indexed by PC1 scores) against latitude for *Cynopterus sphinx* ($n = 251$) sampled from peninsular India.

Microsatellite variation

For the total sample of *C. sphinx* ($n = 251$), mean number of alleles per locus was 11.7 (range = 5–21) and mean observed heterozygosity was 0.73 (range = 0.54–0.79; Table 1). The average within-population inbreeding coefficient was not significantly different from zero ($F_{IS} = 0.027$, 95% CI = −0.004–0.055). Randomization tests revealed a deficit of heterozygotes relative to Hardy–Weinberg expectations in two of the 48 locus × locality combinations (Table 2). However, no F_{IS} values remained significant at a Bonferroni-adjusted α-level of 0.0010. There was no significant correlation between gene diversity ($\arcsin\sqrt{H}$) and latitude ($r = -0.556$, $P = 0.152$). The analysis of microsatellite variation revealed a significant degree of genetic subdivision ($F_{ST} = 0.030$, 95% CI = 0.013–0.045; Table 1). Overall estimates of F_{ST} and R_{ST} were closely similar in magnitude (0.030 vs. 0.033), but pairwise R_{ST} values were characterized by a much higher variance. Results of the coalescent 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.

Table 1 Patterns of variation at six microsatellite loci used in the genetic analysis of *Cynopterus sphinx* from peninsular India

Locus	N_A	H_O	H_E	F_{IS} (= f)	F_{IT} (= F)	F_{ST} (= θ)	R_{ST} (= ρ)
Locus 3	13	0.78	0.83	0.043	0.063	0.021	0.016
CSP-1	12	0.76	0.85	0.058	0.124	0.070	0.003
CSP-2	6	0.73	0.71	−0.012	−0.011	0.000	0.009
CSP-5	13	0.79	0.79	−0.030	0.002	0.031	0.062
CSP-7	21	0.77	0.85	0.056	0.095	0.042	0.102
CSP-9	5	0.54	0.58	0.056	0.069	0.014	0.006
Mean/ total	11.7	0.73	0.77	0.027	0.057	0.030	0.033
UL				0.055	0.092	0.045	0.067
LL				−0.004	0.016	0.013	0.001

N_A, number of alleles per locus; H_O, observed heterozygosity; and H_E, expected heterozygosity. F_{IS} is defined as the average allelic correlation within individuals relative to local populations and F_{IT} represents the corresponding allelic correlation relative to the total array of populations. F_{ST} and R_{ST} are measures of genetic divergence based on the infinite alleles model and stepwise mutation model, respectively. Ninety-five per cent confidence intervals for F statistics and R_{ST} were obtained by bootstrapping over loci (UL = upper limit and LL = lower limit). All 251 bats were genotyped at each locus. Locus 3 and CSP-7 are dinucleotide repeats, CSP-1 and CSP-2 are trinucleotide repeats, and CSP-5 and CSP-9 are tetranucleotide repeats. For primer sequences and PCR protocols, see Storz (2000) and Storz *et al*. (2000b).

Table 2 Locus-specific summary statistics for eight populations of *Cynopterus sphinx* sampled in peninsular India

	Locus					
Pop.	Locus 3	CSP-1	CSP-2	CSP-5	CSP-7	CSP-9
Pune (n = 61)						
N_A	8	9	5	9	12	4
H	0.771	0.698	0.736	0.726	0.812	0.489
F_{IS}	0.121	0.053	0.057	0.001	0.066	−0.022
P	0.052	0.257	0.267	0.548	0.166	0.645
Kolhapur (n = 34)						
N_A	10	10	5	7	11	5
H	0.775	0.887	0.709	0.777	0.859	0.586
F_{IS}	0.013	0.105	−0.119	−0.022	0.110	0.097
P	0.521	0.082	0.918	0.683	0.087	0.271
Belgaum (n = 29)						
N_A	9	9	6	6	12	4
H	0.805	0.810	0.703	0.757	0.864	0.683
F_{IS}	−0.113	−0.021	0.117	−0.002	0.082	0.091
P	0.961	0.682	0.210	0.587	0.198	0.282
Shimoga (n = 25)						
N_A	8	8	4	7	10	4
H	0.833	0.827	0.707	0.820	0.759	0.434
F_{IS}	−0.008	0.178	−0.131	−0.073	−0.212	−0.013
P	0.627	0.052	0.903	0.852	0.999	0.649
Thithimathi (n = 17)						
N_A	7	7	4	9	7	4
H	0.829	0.860	0.726	0.827	0.767	0.660
F_{IS}	0.078	0.316	−0.053	−0.067	0.002	0.109
P	0.333	0.007	0.738	0.833	0.616	0.340
Mysore (n = 23)						
N_A	9	8	4	5	10	4
H	0.837	0.780	0.703	0.688	0.834	0.624
F_{IS}	0.013	0.052	−0.052	−0.075	0.166	0.094
P	0.533	0.394	0.724	0.800	0.055	0.331
Metupalayam (n = 30)						
N_A	9	9	4	10	10	5
H	0.867	0.846	0.699	0.855	0.791	0.589
F_{IS}	0.000	−0.024	−0.144	−0.052	0.031	0.264
P	0.579	0.708	0.925	0.836	0.441	0.030
Othakadai (n = 31)						
N_A	10	10	5	8	11	4
H	0.851	0.860	0.707	0.758	0.826	0.621
F_{IS}	0.128	−0.051	0.087	−0.021	0.102	−0.091
P	0.072	0.843	0.275	0.686	0.121	0.854

N_A, number of alleles; H, Nei's (1987) gene diversity; F_{IS}, within-population inbreeding coefficient; and P, proportion of 10 000 randomizations that yielded F_{IS} values larger (more positive) than those observed. The number of bats sampled from each population is given parenthetically in the leftmost column. All 251 bats were genotyped at each locus.

Interpreting geographical and environmental distance

The first axis of the principal components analysis on temperature variables (PC1-T) explained 86.3% of the variance among localities (eigenvalue = 4.32). Factor loadings were high and positive for each variable (mean annual temperature, r = 0.988; mean maximum daily temperature, r = 0.882; mean minimum daily temperature, r = 0.960; mean relative humidity, r = 0.980), except for mean annual range of temperature (an index of seasonality), which was strongly negative (r = −0.824). PC1-T was therefore interpreted as an overall temperature/equability vector. The first axis of the principal components analysis on rainfall variables (PC1-R) explained 71.4% of the variance among localities (eigenvalue = 2.14). Factor loadings were uniformly high and positive (mean annual rainfall, r = 0.906; mean maximum rainfall, r = 0.996; mean minimum rainfall, r = 0.573). PC1-R was therefore clearly interpretable as an overall rainfall vector. Whereas PC1-R scores exhibited no significant association with latitude (r = 0.294, P = 0.479), PC1-T scores exhibited a strong negative correlation (r = –0.824, P = 0.003). These results are consistent with a previous analysis of latitudinal variation in climatic factors across peninsular India (Storz *et al*. 2001a). Principal components analysis of climatic variables thus produced an ordination of sampling localities across a north-to-south gradient of increasing minimum, maximum, and mean temperature, increasing relative humidity, and decreasing seasonality.

Since PC1-T scores were negatively correlated with latitude, and because sampling localities were distributed along a latitudinal transect, dissimilarity in temperature and humidity conditions increased as a positive function of distance. Thus, matrices of geographical and environmental (PC1-T) distance measures were highly correlated (PC1-T and ln-distance, r = 0.607). With the exclusion of the Thithimathi–Mysore comparison, geographical distance among sampling localities can thus be considered a proxy measure of latitudinally ordered environmental variation. Analysis of PC1-T and PC1-R permitted an assessment of the relative importance of two different sources of environmental variation in relation to clinal size variation in *C. sphinx*.

Relative magnitudes of phenotypic and genetic divergence

Pairwise estimates of F_{ST} and R_{ST} exhibited a monotonic increase as a positive function of distance (Fig. 3). Matrix randomization tests revealed a statistically significant relationship between arcsin $\sqrt{F_{ST}}$ and ln-distance (r = 0.533, P = 0.008) but not for arcsin $\sqrt{R_{ST}}$ and ln-distance (r = 0.226, P = 0.255). Absolute standardized residuals from a regression of arcsin $\sqrt{F_{ST}}$ against ln-distance also exhibited a significant correlation with ln-distance (r = 0.401, P = 0.041). The correlation between residual R_{ST} and distance was not

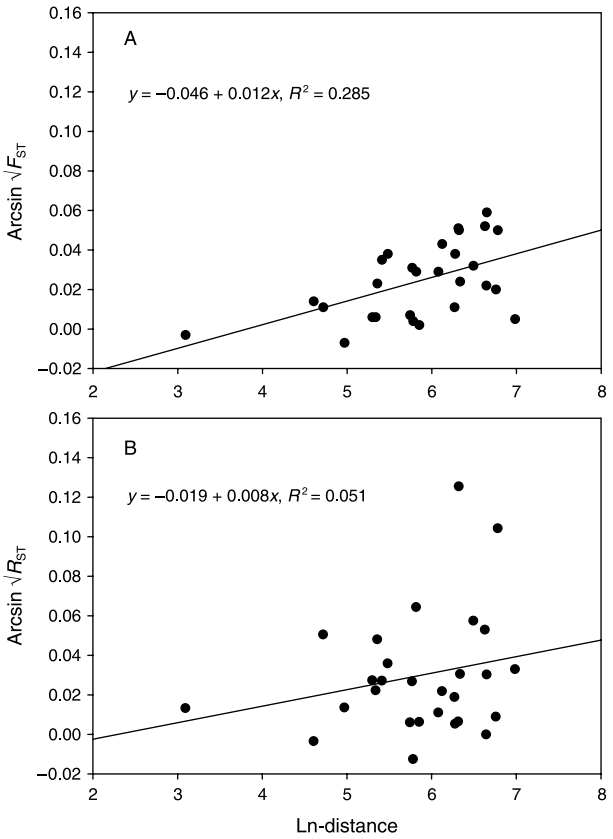

Fig. 3 Least-squares linear regression of (A) arcsin $\sqrt{F_{ST}}$ and (B) arcsin $\sqrt{R_{ST}}$ against ln-distance for each pairwise combination of populations. As a result of the nonindependence of pairwise comparisons and heterogeneity in error variance, statistical significance of regression coefficients was assessed using a matrix randomization test.

statistically significant ($r = 0.272$, $P = 0.139$). The higher variance for R_{ST} relative to F_{ST} agrees with results of simulation studies (Slatkin 1995; Balloux *et al*. 2000). For reasons explained in the Discussion, only estimates of F_{ST} were considered in further analyses.

In the analysis of geographical variation in PC1 scores, there was a significant added variance component in nearly all pairwise comparisons that included one of the three northernmost populations (Table 3). The same pairwise comparisons were also characterized by the largest disparities between Q_{ST} and F_{ST} (Table 4). 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; Table 4). 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 (Fig. 4).

Pairwise MCTs revealed highly significant correlations between Q_{ST} and the following variables: F_{ST}, geographical distance, and the environmental distance based on the temperature/equability vector (PC1-T; Table 5). 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 for the matrices of geographical and environmental distance remained highly significant in the partial MCTs. In other words, the increase in Q_{ST} as a positive function of geographical and environmental distance remained statistically significant even when the effects of neutral divergence (F_{ST}) were partialled out. Results of the partial MCTs were almost identical when a matrix of pairwise R_{ST} was used instead of F_{ST} as a measure of neutral genetic divergence.

In the restricted randomization tests, matrix permutations were restricted to localities within each of two groups: one consisting of the three northernmost localities and one consisting of all remaining localities to the south. This north–south subdivision of the transect was used because of the especially pronounced disparity between Q_{ST} and F_{ST} in pairwise comparisons that included one of the three northernmost populations (Table 4). In these tests the regression coefficients for the matrices of geographical and environmental distance remained significantly greater than zero ($P < 0.05$) even after allowing for overall differences between northern and southern subdivisions of the transect. Thus, results of the restricted randomization tests indicate that the regression coefficients reflect real associations and cannot be explained as artefacts of spatial autocorrelation.

Discussion

In studies of geographical variation, the scope of inference about the interplay between different evolutionary forces is greatly enhanced when patterns of genetically based trait variation are considered in conjunction with estimates of neutral genetic divergence (Lynch *et al*. 1999; Merilä & Crnokrak 2001; McKay & Latta 2002). For example, a matrix of pairwise estimates of neutral genetic divergence can be used as an independent variable in a partial MCT when the observed pattern of phenotypic divergence is tested against causal hypotheses. This general approach has been used previously to disentangle the effects of phylogenetic history and local adaptation in shaping patterns of morphological variation in island lizards (Thorpe 1996; Thorpe *et al*. 1996; Gübitz *et al*. 2000; Malhotra & Thorpe 2000). An alternative approach employed here is to use partial MCTs based on dimensionless measures of phenotypic and genetic divergence that are directly comparable (e.g. Q_{ST} for body size and F_{ST} for microsatellites). The advantage of the approach based on Q_{ST} vs. F_{ST} contrasts is that results can be interpreted within the framework of the neutral theory of phenotypic evolution (Lande 1976, 1977, 1992; Chakraborty & Nei 1982; Rogers & Harpending 1983; Lynch & Hill 1986; Lynch 1988, 1994; Whitlock 1999). This approach provides a means of testing long-standing

Table 3 Matrix of Var(*w*) (above diagonal) and Var(*b*) (below diagonal) for each pairwise combination of populations

	PUN	KOL	BEL	SHI	THI	MYS	MET	OTH
PUN	—	1033.635	1223.922	1246.217	1145.269	1090.927	1210.549	1036.261
		(47.656)	(107.436)	(486.539)	(419.333)	(351.002)	(443.301)	(590.373)
KOL	***333.589***	—	986.216	1001.883	807.904	762.775	971.039	727.526
	(147.662)		(12.346)	(240.015)	(201.463)	(115.844)	(202.825)	(322.819)
BEL	***728.664***	–70.922	—	1325.366	1162.583	1069.848	1261.750	986.955
	(180.458)	(171.678)		(160.334)	(99.231)	(84.679)	(137.546)	(233.565)
SHI	***3227.336***	***1336.345***	***848.406***	—	1204.235	1097.390	1304.407	1006.834
	(187.874)	***(179.944)***	***(250.471)***		(3.487)	(3.486)	(0.150)	(8.616)
THI	***2652.094***	***1046.831***	**486.129**	−16.356	—	859.255	1140.351	792.402
	(181.083)	***(155.481)***	**(237.311)**	(256.744)		(37.587)	(12.183)	(18.120)
MYS	***2301.672***	***634.501***	**440.007**	17.428	−172.246	—	1052.242	747.809
	(166.365)	***(139.263)***	**(205.892)**	(219.478)	(187.505)		(2.996)	(34.940)
MET	***3022.911***	***1173.933***	***759.625***	−0.801	−60.301	15.710	—	973.550
	(177.524)	***(167.770)***	***(228.467)***	(244.338)	(230.386)	(200.655)		(12.843)
OTH	***4047.390***	***1882.339***	***1300.432***	46.400	90.599	**184.884**	72.081	—
	(151.154)	***(124.770)***	***(177.262)***	(186.964)	(158.480)	**(141.323)**	(173.462)	

Standard errors for estimates of variance components are given in parentheses. Var(*w*) and Var(*b*) values are method of moments estimates of the within- and between-population variance of PC1 scores, respectively (see text for details). Because mean squares from ANOVA are unbiased estimators of variance components, Var(*b*) values may be negative when the parametric variance is close to zero. When there is no added variance component between populations, $MS_{between}$ is equally likely to be slightly less than or greater than the error mean square (MS_{within}). Var(*b*) values in bold represent comparisons that revealed a significant added variance component between populations (i.e. when $MS_{between}/MS_{within}$ exceeded the one-tailed critical value of the *F*-distribution at $\alpha = 0.05$). Bold values in italics remained statistically significant at $\alpha = 0.00179$ (Bonferroni-adjusted for 0.05/28 comparisons). Three letter abbreviations correspond to sampling localities listed in the legend for Fig. 1.

hypotheses about the ecological causes of population differentiation and the role of selection in maintaining clinal variation.

Isolation by distance

The spatial patterning of pairwise F_{ST} satisfied two criteria that can be used to determine whether a regional population has attained migration–drift equilibrium: (i) a significant association between pairwise F_{ST} and distance, and (ii) a scatterplot of pairwise F_{ST} vs. distance that reveals a positive and monotonic relationship over the full range of distance values (Hutchison & Templeton 1999). The first pattern is indicative of isolation-by-distance, and the second pattern reflects the fact that random fluctuations in allelic frequencies increase when the homogenizing effect of gene flow is attenuated between widely separated populations. Thus, the spatial patterning of microsatellite variation indicates that *Cynopterus sphinx* has attained migration–drift equilibrium under high levels of gene flow across peninsular India.

Genetic evidence suggests that *C. sphinx* has undergone a population contraction in the Indian subcontinent, possibly as a result of climatically induced range shifts during the late Pleistocene or early to mid-Holocene (Storz & Beaumont 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 & Templeton 1999). However, because the observed pattern of microsatellite variation in *C. sphinx* is 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.

Q_{ST} *vs.* F_{ST}

As illustrated in Fig. 4, patterns of geographical divergence in body size (revealed by Q_{ST}) and microsatellite markers (revealed by F_{ST}) are highly discordant. Results of the partial MCTs 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 north-to-south gradient of increasing temperature, increasing relative humidity, and decreasing seasonality. The geographical patterning of pairwise Q_{ST} is most likely attributable to spatially varying selection and / or the direct influence of latitudinally ordered environmental effects. However, before invoking selection to explain the

Table 4 Matrices of (A) straight-line separation distances (km), (B) Q_{ST}, (C) F_{ST} (= θ), and (D) R_{ST} (= ρ) for each pairwise combination of populations

	PUN	KOL	BEL	SHI	THI	MYS	MET
(A)							
KOL	224						
BEL	320	100					
SHI	556	336	240				
THI	756	532	436	200			
MYS	772	552	456	212	22		
MET	880	660	564	324	144	112	
OTH	1080	860	768	528	348	312	208
(B)							
KOL	0.244						
BEL	0.373	−0.077					
SHI	0.721	0.572	0.390				
THI	0.698	0.564	0.295	−0.014			
MYS	0.678	0.454	0.291	0.016	−0.251		
MET	0.714	0.547	0.376	−0.001	−0.056	0.015	
OTH	0.796	0.721	0.569	0.044	0.103	0.198	0.069
(C)							
KOL	0.035						
BEL	0.031	0.014					
SHI	0.050	0.029	0.038				
THI	0.052	0.038	0.029	0.006			
MYS	0.059	0.051	0.043	0.023	−0.003		
MET	0.050	0.032	0.024	0.004	−0.007	0.011	
OTH	0.005	0.020	0.022	0.011	0.002	0.007	0.006
(D)							
KOL	0.027						
BEL	0.027	−0.003					
SHI	0.125	0.064	0.036				
THI	0.053	0.005	0.011	0.027			
MYS	0.030	0.007	0.022	0.048	0.013		
MET	0.104	0.058	0.031	−0.013	0.014	0.051	
OTH	0.033	0.009	0.000	0.019	0.006	0.006	0.022

Using the method of moments (Sokal & Rohlf 1995; Weir 1996), unbiased estimates of variance components were obtained from an ANOVA on PC1 scores (Q_{ST}), allelic frequencies (F_{ST}), or standardized allelic lengths (in repeat units; R_{ST}). When there is no added variance component between populations, $MS_{between}$ is equally likely to be slightly less than or greater than the error mean square (MS_{within}). Thus, unbiased estimates of Q_{ST}, F_{ST}, or R_{ST} may be negative when the parametric value of the between-population variance is close to zero.

observed pattern, it is first necessary to confirm (i) that F_{ST} for microsatellites provides an unbiased benchmark for the null expectation of neutral divergence and (ii) that Q_{ST} accurately reflects the partitioning of additive genetic variance in body size. Each of these issues will be discussed in turn.

Microsatellites and F_{ST}

High mutation rates characteristic of microsatellite loci may produce a systematic downward bias in F_{ST} estimates (Charlesworth 1998; Nagylaki 1998; Flint *et al*. 1999; Hedrick 1999; Balloux *et al*. 2000). Under equilibrium conditions in an island model of population structure, and assuming that mutational dynamics conform to the infinite alleles model, Wright (1969; p. 291) demonstrated that $F_{ST} = (1 + 4N_e m + 4N_e \mu)^{-1}$, where N_e = effective size of local populations, m = migration rate, and μ = mutation rate. When the mutation rate is negligibly small relative to the migration rate, F_{ST} is a simple function of the number of migrants ($N_e m$; Crow & Aoki 1984; Cockerham & Weir 1993; Neigel 1997). However, depending on the dispersal ability of the species under consideration and the spatial scale at which divergence is being assessed, the mutation rate for microsatellite loci may not be negligibly small relative to migration (Balloux *et al*. 2000). This problem is further exacerbated if mutational dynamics generate allelic size homoplasy, as expected under the stepwise mutation model (Slatkin 1995; Rousset 1996).

Table 5 Results of pairwise (A) and partial (B) matrix correspondence tests of causal hypotheses regarding the pattern of clinal size variation in *Cynopterus 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 matrix correspondence tests (MCTs) 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.

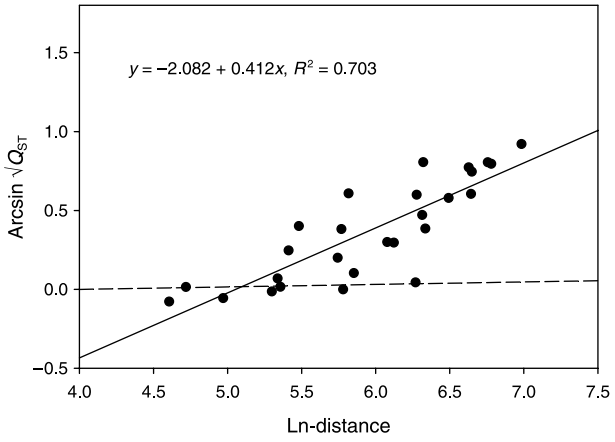

Fig. 4 Least-squares linear regression of arcsin $\sqrt{Q_{ST}}$ against ln-distance for each pairwise combination of populations. The dashed line denotes the linear regression line for arcsin $\sqrt{F_{ST}}$ vs. distance (note difference in scale of the *y*-axis compared to Fig. 3). Statistical significance of the regression coefficient was assessed using a matrix randomization test.

However, the mode of microsatellite mutation should not affect the interpretation of F_{ST} in this study. Having verified that *C. sphinx* is at migration–drift equilibrium across the region surveyed (see above), the estimate of F_{ST} for the two most widely separated populations (= 0.005) translates into $N_e(m + \mu) = 49.75$ using Wright's island model approximation. The northern population in this comparison (Pune) has an estimated effective size of 108.2 ($N_e/N = 0.42$, average $N = 257.5$ adults; Storz *et al*. 2001c). Thus, even for mutation rates as high as 1×10^{-3}–1×10^{-4} per haploid gamete per generation (Ellegren 2000), the gametic migration rate would still have to be several orders of magnitude larger to account for the F_{ST}-based estimate of $N_e(m + \mu)$. Across the spatial scale considered in this study, rates of migration are so high relative to possible rates of mutation that Weir & Cockerham's (1984) unbiased estimator of $F_{ST}(= \theta)$ can be expected to provide a more accurate measure of genetic divergence than statistics based on the stepwise mutation model (Slatkin 1995; Gaggiotti *et al*. 1999). It remains true that high levels of within-population variation can place an upper limit on the maximal value of F_{ST} (Hedrick 1999). However, the magnitude of the expected reduction in F_{ST} for highly variable loci (Flint *et al*. 1999) is not sufficient to explain the profound discrepancies between Q_{ST} and F_{ST} observed in this study.

Quantitative traits and Q_{ST}

Whitlock (1999) demonstrated that Q_{ST} for a neutral quantitative trait with an additive genetic basis is equal to F_{ST} for a neutral locus in the limit of low mutation rates. This result is quite general for all modes of population structure (see also Lynch 1994). This result lends general support to the use of Q_{ST} vs. F_{ST} contrasts as a means of testing the neutral model of phenotypic divergence (Rogers & Harpending 1983; Lewontin 1984; Felsenstein 1986; Rogers 1986; Lande 1992; Prout & Barker 1993; Spitze 1993). An important caveat is that statistical properties of variance ratios such as Q_{ST} and F_{ST} are not well understood, and estimation error is likely to be quite large.

It should be noted that the Q_{ST} vs. F_{ST} neutrality test is only valid when Q_{ST} is calculated from trait values. Unless the trait in question is selectively neutral, Q_{ST} is not equivalent to F_{ST} for allelic variation at quantitative trait loci

(QTLs) underlying the trait (Latta 1998). Selection on quantitative traits has a much greater effect on the covariance of allelic frequencies (the between-population component of linkage disequilibrium; Ohta 1982) than on the variance in allelic frequencies among populations. When spatially varying selection is strong (i.e. disparity in trait optima between populations is large) and the rate of gene flow is high, trait divergence may occur as a result of the covariance of allelic effects, even in the absence of appreciable shifts in allelic frequencies at the underlying QTLs. Allelic frequencies of QTLs will depart from the neutral expectation of migration–drift equilibrium only if differences between the variance in trait optima and the between-population variance due to drift ($2F_{ST}\,\sigma_0^2$) exceeds the maximum covariance of allelic frequencies (Latta 1998).

When Q_{ST} for a particular trait is significantly greater than F_{ST} for neutral markers (a commonly observed pattern; Lynch *et al*. 1999; Merilä & Crnokrak 2001; McKay & Latta 2002), it is important to confirm that Q_{ST} estimates are not inflated by nonadditive genetic effects (dominance and/or epistasis) or environmental effects. Theoretical results suggest that dominance and epistasis are unlikely explanations for the observed disparity between Q_{ST} and F_{ST}. The contribution of dominance variance to Q_{ST} approaches zero when averaged over a uniform distribution of allelic frequencies (Whitlock 1999). However, the effect of interactions among loci with different allelic frequencies and dominance relationships remains poorly understood. Lynch *et al*. (1999) suggested that epistatic variance may inflate estimates of Q_{ST}. However, as demonstrated by Whitlock (1999), additive-by-additive epistasis results in an increase in the between-population component of variance on an absolute scale, but Q_{ST} is actually reduced relative to expectations of the neutral additive model.

Inferences about the adaptive basis of clinal variation are strengthened when the environmental component of phenotypic variation can be identified and statistically removed (Coyne & Beecham 1987; Mousseau & Roff 1989, 1995; Long & Singh 1995; Huey *et al*. 2000). This can be accomplished by comparing different populations using 'common-garden' or reciprocal transplant experiments (Mousseau 1999). Unfortunately, this approach is not practical for many vertebrate species. Provided that certain restrictive conditions apply, a possible alternative is to use genetic marker-inferred relatedness to estimate variance components in free-ranging populations (Mousseau *et al*. 1998; Ritland 1999; 2000). In this study, within-population phenotypic variance ($\times 0.5$) was used as a proxy for additive genetic variance. A similar approach has been used to assess the role of selection and/or environmental effects in determining geographical patterns of morphometric variation in human populations in the Solomon Islands (Rogers & Harpending 1983) and populations of the greenfinch (*Carduelis chloris*) in continental Europe (Merilä 1997).

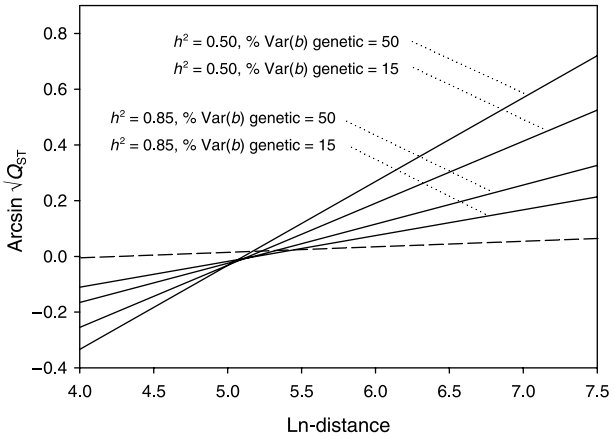

Fig. 5 Least-squares linear regression lines for arcsin $\sqrt{Q_{ST}}$ against distance. For each linear regression, Q_{ST} was recalculated using a range of values for Var(*w*) (assuming h^2 in the range 0.50–0.85) and Var(*b*) (assuming that 50–85% of the between-population variance was attributable to a nonheritable environmental component). The dashed line denotes the linear regression line for arcsin $\sqrt{F_{ST}}$ vs. distance (note difference in scale of the *y*-axis compared to Fig. 3).

Although the polygenic basis of mammalian body size has been well characterized (Falconer & Mackay 1996), ecological considerations suggest that size may be particularly responsive to environmental induction (phenotypic plasticity). For example, variation in postnatal growth rate and onset of sexual maturity can both exert a strong influence on adult body size.

To examine the effects 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 $\sqrt{Q_{ST}}$ vs. ln-distance remained significantly steeper than that for arcsin $\sqrt{F_{ST}}$ vs. ln-distance (Fig. 5). 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 MCTs between the recalculated Q_{ST} matrix and each of the geographical and PC1-T distance matrices remained statistically significant. In conclusion, 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.

Although the analysis of purely phenotypic data can provide much insight into the evolutionary processes that shape patterns of geographical variation (e.g. Smith *et al*. 1997; Schneider *et al*. 1999), controlled breeding experiments are ultimately needed to assess the relative importance of

genetically and environmentally based variation in trait values. Even in studies that use breeding experiments to measure the narrow-sense heritability of a particular trait, the between-population component of variance is typically estimated from phenotypic trait values alone (i.e. the additive genetic variance is not measured directly). To obtain more refined insights into the evolutionary mechanisms underlying trait divergence, it will be important for future studies to measure directly the additive genetic components of the within- and between-population variance in trait values.

Spatially varying selection, gene flow and clinal variation

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 & 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 geographically as an adaptive response to broad-scale climatic gradients (for review, see Storz *et al*. 2001a). 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*.

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The main focus of the author's current research is molecular population genetics, with an emphasis on understanding the process of adaptive evolutionary change in natural populations.
