Testing for Asymmetrical Gene Flow in a *Drosophila melanogaster* Body-Size Cline

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ABSTRACT

Asymmetrical gene flow is an important, but rarely examined genetic parameter. Here, we develop a new method for detecting departures from symmetrical migration between two populations using microsatellite data that are based on the difference in the proportion of private alleles. Application of this approach to data collected from wild-caught *Drosophila melanogaster* along a latitudinal body-size cline in eastern Australia revealed that asymmetrical gene flow could be detected, but was uncommon, nonlocalized, and occurred in both directions. We also show that, in contrast to the findings of a previous study, there is good evidence to suggest that the cline experiences significant levels of gene flow between populations.

ASYMMETRY in the movement of individuals and genes between populations affects both the ability of populations to adapt locally (*e.g.*, Stearns and Sage 1980; Riechert 1993; Dias *et al.* 1996) and the evolution of species ranges (*e.g.*, García-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997; Barton 2001). However, few studies have examined the extent of asymmetrical gene flow in natural populations, a trend that is at least partly due to a scarcity of diagnostic tests that can be applied to genetic data.

In studies where asymmetrical gene flow has been reported, it has been inferred either from differences in the level of linkage disequilibrium within populations (*e.g.*, Dias *et al.* 1996) or from changes in the shape of a cline over time (*e.g.*, Lenormand and Raymond 2000). Several recent studies have also used the software package MIGRATE (Beerli and Felsenstein 1999, 2001) to estimate the number of migrants (Nm; N is the effective population size and m is the migration rate) into each population per generation from genetic data and hence determine if gene flow has been asymmetrical (*e.g.*, Miura and Edwards 2001; Zheng *et al.* 2003). The MIGRATE program calculates migration parameters, using a maximum-likelihood and coalescent theory approach. It also provides the opportunity to test if migration rates between populations are symmetrical, using a likelihood-ratio test.

Here, we describe a test for detecting deviations from symmetrical gene flow between two populations, using microsatellite data that are based on differences in the proportion of private alleles. The utility of this method is demonstrated by assessing the extent of asymmetrical gene flow in a *Drosophila melanogaster* latitudinal body-size cline on the east coast of Australia (James *et al.* 1995; Gockel *et al.* 2001). Asymmetrical gene flow has been suspected to occur along this cline because body-size differences between populations are much smaller at low latitudes than at high latitudes. This nonlinearity in the cline is thought to reflect higher emigration rates from populations in warmer climates, reducing the magnitude of genetic differentiation between them (James *et al.* 1995). We also examine population structure with the aim of assessing whether or not the cline is being maintained by selection despite high gene flow, a view recently challenged by Agis and Schlötterer (2001), and if the overall levels of gene flow change with latitude.

MATERIALS AND METHODS

Fly collections: Wild *D. melanogaster* were collected from rotting fruits or baited traps at 16 different latitudes from a 3000-km north-south transect along the east coast of Australia during January and February 2000 (Figure 1). This transect includes sites 200 km farther north than the lowest-latitude site collected by James *et al.* (1995). Sampling was structured so that two sites (2.8–50.4 km apart), designated A and B, were sampled within each latitude, and one to two microsites (0.005–0.4 km apart), designated 1 and 2, were sampled within each site. Latitudinal sites varied in spacing along the transect and were densely clustered in a section between 32.6° and 37.5°S, where the steepest slope of body-size change was observed by James *et al.* (1995) and Gockel *et al.* (2001). Up to 10 flies were sampled from each microsite and were either frozen in liquid nitrogen or stored in absolute ethanol until their DNA was extracted.

Microsatellite genotyping: DNA extraction from individual flies, PCR protocols, and allele scoring followed methods outlined in Gockel *et al.* (2001). The loci used in this study are listed in Table 1. Because these loci were specifically selected for a high number of repeat units, some of these markers show variances in repeat number that are unusually high for *D. melanogaster*.

Data analysis: Measures of genetic variation, such as expected heterozygosity and the variance in repeat number, were calculated for each site using the MICROSAT v1.4 software package (Minch *et al.* 1995). Population structure was assessed using hierarchical analysis of molecular variance (AMOVA)

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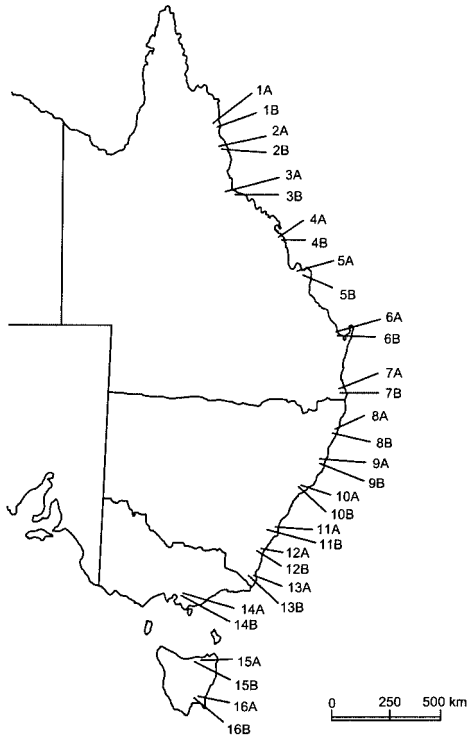

FIGURE 1.—Map of the eastern coast of Australia and the sampled sites.

with the ARLEQUIN software package (SCHNEIDER *et al.* 2000). Pairwise θ values, an unbiased estimate of F_{ST} (WEIR and COCKERHAM 1984), were also calculated over all loci with the FSTAT software package (GOUDET 1995).

The significance of pairwise θ values, hereafter referred to as pairwise F_{ST}, was tested by permuting genotypes rather than alleles among samples, as this is the preferred method when Hardy-Weinberg is rejected within samples (GOUDET 1995), and was corrected for multiple comparisons using sequential Bonferroni procedures. To test for a linear association between genetic and geographical distance between sites, we compared an $F_{ST}/(1 - F_{ST})$ matrix with a geographical distance matrix (natural log kilometers; ROUSSET 1997), using the Mantel test (10,000 permutations) in the GENEPOP program (RAYMOND and ROUSSET 1995).

Spatial patterns of variation at each locus were summarized by *II* values, a spatial autocorrelation statistic designed specifically for DNA data (BERTORELLE and BARBUJANI 1995a), by comparing all possible pairs of chromosomes using the AIDA computer program. These comparisons were carried out at arbitrary distance classes, *e.g.*, at distances of 0 (within a sampling site), between 0.01 and 500 km, between 501 and 1000 km, etc. *II* is a product-moment coefficient, analogous to Moran's *I* that quantifies both allele length and frequency differences among localities. It may vary between −1 and +1 and has an expectation close to zero when alleles are randomly distributed (BERTORELLE and BARBUJANI 1995a). Positive *II* values indicate overall similarity between samples at that distance, and negative values indicate dissimilarity. The statistical significance of *II* values at each distance class was assessed by comparison to a null distribution of *II* values that assumes chromosomes are randomly distributed in space. This randomization process was repeated 2000 times for each locus and allowed for a level of significance up to 0.001 (two-tailed test) to be tested.

Tests for asymmetrical migration between adjacent populations: We based our test of asymmetrical migration on the idea that asymmetry in gene flow between two populations will lead to asymmetry in the proportion of private alleles (number of unique alleles/total number of alleles). To explore this further, we carried out forward simulations that

TABLE 1

Details and variability of the 20 microsatellite loci used

Locus	Genetic location	No. of alleles[a]	Variance in repeat no.	Expected heterozygosity
AC004373	2-12.5	11 (174–220)	2.3	0.68
AC004721	2-17.5	12 (186–214)	2.3	0.59
AC003052	2-22	16 (253–293)	5.5	0.74
AC005555	2-31.5	12 (130–178)	10.6	0.79
AC005889	2-35	11 (183–215)	3.4	0.67
AC005115	2-44	10 (172–222)	3.2	0.48
AC004759	2-54.3	10 (217–248)	2.8	0.59
AC005463	2-58	10 (167–219)	4.3	0.75
DMMP20	2-68	10 (79–101)	3.6	0.43
AC004516	2-76	20 (243–289)	13.9	0.85
AC004641	2-81	27 (217–275)	24.0	0.92
AC004365	2-97.5	16 (187–229)	8.9	0.76
AC004343	3-0.5	12 (190–236)	3.1	0.68
AC004658	3-8	12 (110–134)	5.7	0.70
AC004767	3-16.5	17 (277–333)	26.2	0.78
DMU14395	3-18	21 (262–310)	7.4	0.75
DROPROSA	3-51	11 (108–130)	7.4	0.73
DMU25686	3-73	16 (130–160)	15.6	0.79
AC008193	3-77	18 (188–230)	21.6	0.85
DMU1951	3-81	14 (176–214)	7.3	0.88

[a] Minimum and maximum allele sizes (bp) are in parentheses.

were based on a model that portrays two populations capable of exchanging migrants each generation. Individuals were haploid and the number of individuals within each population was held constant. Populations were founded with no genetic variation. Generations were nonoverlapping and had the following steps: mutation, Wright-Fisher sampling, and migration. The mutation process followed a strict stepwise model and allele size was unconstrained.

We ran a variety of models with different numbers of loci, levels of asymmetrical migration, and numbers of migrants. Each model ran for 2000 generations and each was repeated 1000 times. We found that, with equal population sizes, most distributions of the difference in proportion of private alleles (δPPA) between populations were significantly different from zero [*i.e.*, zero was outside the 95% confidence limits (CL)] when migration was unidirectional and the number of loci was at least 10 (data not shown). However, with more modest levels of asymmetry (<4:1), the differences between populations became less, and the distributions were not significantly different from zero. Even so, in all cases of asymmetrical migration, the mean of the distribution was negative, indicating that the proportion of private alleles was higher, on average, in the population receiving more migrants. The CL were sensitive to both the number of loci and the number of migrants each generation, increasing in range as both these parameters decreased.

On the basis of these results, we developed a test to detect asymmetrical migration between two populations by calculating the CL for a null model that assumed symmetrical migration. From earlier simulations we had found that the magnitude of the CL was dependent on the product of the population size (N) and mutation rate (μ), but not on N and μ independently. It was also apparent that the CL stabilized relatively soon after populations were initiated, long before they had reached mutation-drift equilibrium (Figure 2). Since the variance in repeat number is an estimator of $N\mu$ (Slatkin 1995; Kimmel *et al.* 1996), we used the observed repeat variance in each pair of sites to estimate the corresponding CL. A null hypothesis of symmetrical migration between the two sites was rejected if the observed δPPA fell outside the 95% CL.

To calculate δPPA between adjacent sites we split the sites into two groups, A and B sites (the northern- and southernmost sites at each latitude, respectively). This ensured that there was an independent set of site comparisons for each pair of adjacent latitudes. In this way we could assess whether any deviations from symmetrical migration observed along the cline were systematic or not. In cases where there was a difference in the number of microsites sampled in a site comparison (*i.e.*, one *vs.* two or vice versa), then only one microsite from each site was used. To further reduce any potential bias due to differences in sample size, we resampled the minimum number of alleles at each locus with replacement for each site comparison 100 times. The proportion of private alleles in each locus was calculated by dividing the total number of private alleles by the total number of alleles.

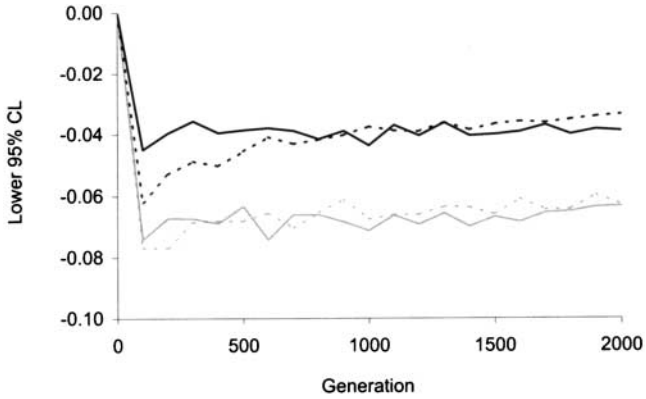

Figure 2.—The lower 95% confidence limits (CL) of the difference in the proportion of private alleles (δPPA) between two populations exchanging equal numbers of migrants each generation. CL were calculated from 1000 independent simulations. Solid lines represent models where $N = 100$ and $\mu = 0.05$ and dashed lines models where $N = 1000$ and $\mu = 0.005$. Black lines represent models with migration at 10 individuals per generation and gray lines models with migration at 1 individual per generation.

RESULTS AND DISCUSSION

Variation within sites: Expected heterozygosity and variance in repeat number, both averaged over loci, ranged from 0.59 to 0.70 and 5.9 to 10.9, respectively. On average, these estimates were 1.3 (expected heterozygosity) and 2.8 (repeat variance) times higher than those reported in a recent microsatellite survey of isofemale lines from eastern Australia (Agis and Schlötterer 2001). The increased variation within sites reported here most likely reflects the higher variability of the loci selected, but might also reflect our use of wild-caught flies rather than laboratory isofemale lines. Both measures of variability were negatively associated with latitude, but only expected heterozygosity had a significant linear relationship ($R^2 = 0.42$, $P < 0.001$ and $R^2 = 0.09$, $P = 0.098$ for expected heterozygosity and repeat variance, respectively). Estimates of expected heterozygosity and variance in repeat number reported by Agis and Schlötterer (2001) show similar negative association with latitude ($R^2 = 0.90$, $P < 0.001$ and $R^2 = 0.38$, $P = 0.077$ for expected heterozygosity and repeat variance, respectively). Hence, the higher variation within sites reported here may also reflect our sampling of the entire cline rather than sites at middle and high latitudes as in the earlier study.

The higher levels of variability observed in low-latitude sites might reflect larger effective population sizes or be a consequence of colonization history. In agreement with the second hypothesis, Zwaan *et al.* (2000) have suggested that the cellular basis of the change in body size along the cline is indicative of a southward expansion of *D. melanogaster* in eastern Australia. However, we should note that it is unlikely that Australia was colonized directly from East Asia, as there is both morphological (David and Capy 1988) and molecular evidence (Hale and Singh 1991) to suggest an Afro-European origin of Australian populations. On the basis of earliest reports of *D. melanogaster* in Australia (see Bock and Parsons 1981) it is known that colonization occurred <100 years ago.

Population structure: Pairwise population differentiation tests revealed that there was no significant genetic differentiation between microsites at any one site (*i.e.*,

TABLE 2

Spatial autocorrelation analysis of microsatellite variation

	Distance class limits (km)						
Locus	0	0.01–500	501–1000	1001–1500	1501–2000	2001–2500	>2500
AC004373	1.93**	−0.22	0.02	−0.21	0.12	−0.51*	−0.10
AC004721	4.23***	0.64**	−0.04	−0.02	−1.07***	0.00	−2.13***
AC003052	8.72***	0.82***	0.05	−0.64*	−1.69***	−0.86*	0.62*
AC005555	2.72***	1.52***	0.35**	−0.04	−0.51	−2.04***	−3.66***
AC005889	0.89	0.32*	−0.15	−0.35	−0.24	0.29	−1.29**
AC005115	1.23	1.14***	−0.09	−0.34	−1.62***	−0.24	0.42
AC004759	5.84***	2.35***	0.69***	0.19	−1.33***	−2.77***	−6.16***
AC005463	7.02***	1.96***	0.22	−2.03***	−3.00***	−0.63	4.51***
DMMP20	10.05***	2.78***	0.62***	−0.43	−1.37***	−4.96***	−2.56***
AC004516	1.49*	0.63*	0.41**	−0.23	−0.60*	−1.25**	−1.16*
AC004641	7.56***	0.53**	0.83***	−0.46*	0.25*	−2.84***	−2.94***
AC004365	5.45***	−0.04	0.36**	−0.73*	−0.29	−0.20	−1.23*
AC004343	3.15***	2.27***	0.91***	−1.52***	−2.42***	−2.22***	1.84***
AC004658	3.21***	0.26	0.09	−0.41	−0.36	−0.15	−1.39*
AC004767	6.33***	0.30	−1.34***	0.91***	−1.19***	0.79**	−0.32
DMU14395	3.85***	1.12***	0.30**	−0.07	−0.55*	−2.02***	−3.61***
DROPROSA	1.05	0.48*	−0.04	−0.75**	−0.40	−0.23	0.65*
DMU25686	10.95***	6.75***	2.35***	−0.62*	−3.90***	−10.58***	−8.20***
AC008193	12.52***	7.81***	3.81***	−0.38	−2.84***	−10.97***	−20.17***
DMU1951	4.26***	1.34***	0.59***	−0.00	−0.73**	−2.44***	−3.87***

Values are ×100. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

over distances of 0.005–0.4 km) or between sites within latitudes after microsite data within each site had been pooled (*i.e.*, over distances of 2.8–50.4 km, data not shown). This was confirmed with AMOVA, which revealed no significant genetic structure between microsites within sites or between sites within latitudes when microsites were pooled (variance components were negative and 0.28% of the total variance, respectively). However, there was a low, but significant level of population substructuring between latitudes (variance component = 2.19%; $P < 0.001$).

As expected, the level of population differentiation increased with the geographic distance between sites (Mantel test, $P < 0.001$). Interestingly, and in contrast to Agis and Schlötterer (2001), this relationship was also significant when Tasmanian populations were excluded and when the northern and southern halves of the cline were analyzed separately (Mantel tests, $P < 0.001$ in all cases). This discrepancy may reflect the small number of mainland sites (seven) sampled by Agis and Schlötterer (2001) or result from using wild-caught flies rather than isofemale lines that have been in laboratory culture for several years.

The apparent absence of an effect of geographic distance on genetic differentiation on the Australian mainland led Agis and Schlötterer (2001) to conclude that the observed differences between populations reflect variation in effective population sizes rather than patterns of gene flow. Evidently, in light of our results, this conclusion requires reassessment as they suggest gene flow between populations is both apparent and equally strong in the northern and southern halves of the cline.

A second difference from the results of Agis and Schlötterer (2001) was that we found that a significant proportion of pairwise F_{ST} values between Australian mainland and Tasmanian sites were smaller than those between sites within the Australian mainland (data not shown). Again, this discrepancy might be due to differences in the number of sites sampled or be a result of using wild-caught flies rather than isofemale lines. In any event, our result calls into question Agis and Schlötterer's (2001) suggestion that Tasmania was colonized from a different source population than was mainland Australia.

Autocorrelation coefficients for 500-km distance classes are given in Table 2. In most cases *II* values did not exceed 0.1. This reflects the small interpopulational differences along the cline and the high variability within sites. Despite this, most loci showed significant departures from randomness. Overall, a common pattern was shown by most loci whereby *II* values were positive and significant at distance classes up to 500 km and near zero and nonsignificant at intermediate distance classes. Thereafter, two main patterns emerged. The first was that *II* values became increasingly more negative and significant with greater distance (*e.g.*, AC005555, AC004759, DMU14395, DMU25686, AC008193, and DMU1951). This pattern is identical to the one observed for clines in allele frequencies both in simulation (Bertorelle

TABLE 3

Pairwise comparisons of the proportion of private alleles (PPA) between adjacent sites along the cline

	A sites			B sites		
Comparison	PPA_i	PPA_{i+1}	δPPA	PPA_i	PPA_{i+1}	δPPA
1 *vs.* 2	0.183	0.195	−0.012	0.164	0.195	−0.031
2 *vs.* 3	0.244	0.231	0.014	0.203	0.210	−0.007
3 *vs.* 4	0.182	0.227	−0.045	0.196	0.279	−0.083[a]
4 *vs.* 5	0.258	0.325	−0.067[a,b]	0.297	0.209	0.088[a]
5 *vs.* 6	0.313	0.270	0.043	0.222	0.261	−0.039
6 *vs.* 7	0.249	0.257	−0.008	0.233	0.236	−0.003
7 *vs.* 8	0.311	0.263	0.048	0.317	0.278	0.040
8 *vs.* 9	0.331	0.368	−0.037[a]	0.281	0.281	0.000
9 *vs.* 10	0.380	0.366	0.014	0.217	0.303	−0.086[a]
10 *vs.* 11	0.368	0.404	−0.036[a]	0.302	0.306	−0.004
11 *vs.* 12	0.381	0.346	0.035	0.341	0.230	0.111[a,b]
12 *vs.* 13	0.384	0.355	0.030	0.306	0.305	0.001
13 *vs.* 14	0.334	0.398	−0.053	0.323	0.291	0.032
14 *vs.* 15	0.302	0.272	0.029	0.293	0.292	0.001
15 *vs.* 16	0.330	0.260	0.070[a]	0.301	0.241	0.060[a,b]

[a] Comparisons where the null model of symmetric migration with a migration rate of five individuals per generation was rejected.

[b] Comparisons where the null model of symmetric migration with a migration rate of one individual per generation was rejected.

and Barbujani 1995a) and in empirical studies (Chikhi *et al.* 1998). Genetic gradients can be caused by selection, demic diffusion, or a range expansion accompanied by founder effects and gene flow (Bertorelle and Barbujani 1995b). The second pattern was that *II* values started to decrease, but were then followed by upward fluctuations at intermediate and remote distance classes (*e.g.*, AC004721, AC003052, AC005115, AC005463, AC004641, AC004365, AC004343, AC004767, and DROPROSA). Patterns of this kind are referred to as "long distance differentiation" and are regarded as ancient clines on which the effects of successive gene flow, drift, and/or adaptation to local environmental factors have been superimposed (see Bertorelle and Barbujani 1995a,b).

The loci with the most pronounced clinal autocorrelation profiles were DMU25686, AC008193, and DMU1951. These loci occur within the common cosmopolitan inversion *In(3R)Payne*, which is known to vary clinally with latitude on several continents and is hence thought to be under selection (Knibb *et al.* 1981; Anderson *et al.* 1987). Clinal autocorrelation profiles in these loci might therefore be due to alleles hitchhiking with genomic regions under clinal selection. Interestingly, Weeks *et al.* (2002) have recently shown a strong positive association between size and *In(3R)Payne* in a central population within the Australian cline, and Gockel *et al.* (2002) have shown that the right arm of the third chromosome (where this inversion is found) controls a significant proportion of the natural genetic variation in body size. It is therefore possible that selection on the inversion may be directly related to selection on body size, and that this may be influencing patterns of variation at these microsatellite loci.

Clinal autocorrelation profiles at other loci might also be associated with chromosomal inversions under selection. For example, the locus AC005555 is located within the inversion *In(2L)t* and DMU14395 is located within the inversion *In(3L)Payne*. As with *In(3R)Payne*, these inversions vary latitudinally on several continents and are thought to be under clinal selection (Knibb *et al.* 1981; Anderson *et al.* 1987). However, not all loci with clinal autocorrelation profiles were located within known inversions, and not all loci within clinally selected inversions had clinal autocorrelation profiles.

Because parallel patterns of geographic variation at a number of loci are taken as evidence that factors affecting the entire genome are responsible (Sokal 1979), the most likely explanation for the autocorrelation patterns observed in the study is a demographic expansion. This is consistent with the idea that *D. melanogaster* colonized the Australian continent by a southward range expansion accompanied by repeated founder effects, as suggested from the clinal patterns in genetic variability within populations (see above). Gene flow and genetic drift have subsequently broken down the underlying genetic gradient, leading to the long-distance differentiation and shallow clinal patterns seen in most loci. However, a few loci have maintained very steep genetic gradients, and this might be attributed to selection.

Tests of asymmetrical migration: Pairwise comparisons of the proportion of private alleles within adjacent populations along the cline are given in Table 3. Taking

into account differences in the variance in repeat number between sites, there were only nine instances (out of the 30 possible comparisons) where δPPA deviated significantly from a null model of symmetric migration at a level of five individuals (10 gametes) per generation. When the level of symmetric migration was reduced to one individual per generation, there were only three instances where the null model was rejected. Overall, pairwise comparisons that deviated significantly from null models tended to be spread randomly along the cline and were represented equally by positive and negative differences. These results suggest that asymmetrical migration, although detectable, was not a common feature of the cline. They also suggest that asymmetrical migration was not localized or predominately in one direction.

To test the robustness of our results, we applied the MIGRATE 1.6.9 program (Beerli and Felsenstein 2001) to microsatellite data from five pairs of adjacent sites in which the null model of symmetrical migration had been rejected. Analyses were repeated several times for each pair of sites to check the consistencies of the results. We found that in nearly all cases, estimates of *Nm* into each site were significantly different (95% CL were nonoverlapping) and were biased in the same direction as predicted from our tests. Further, hypotheses of equal migration rates between the two sites were rejected. The one exception was a pair of sites in which the number of individuals sampled in one of the sites was much lower than usual (7 compared to 20). In this data set, the results from the MIGRATE program suggested that gene flow between the two sites was symmetrical. Thus it would appear that a degree of caution should be associated with our test when the number of individuals sampled is low. Nonetheless, the overall pattern seems to be that the two methods give similar results.

CONCLUSIONS

As with previous studies, our survey of genetic variation in *D. melanogaster* revealed low, but statistically significant, differentiation among most populations. However, we found no evidence to support Agis and Schlötterer's (2001) view that gene flow among populations was low or that Tasmanian populations were genetically distinct from mainland populations. Instead, our results indicate that gene flow has been sufficient to maintain higher than expected genetic homogeneity between sites up to 500 km apart and has possibly caused fluctuations in underlying clinal patterns at distances >1500 km. Our data therefore support the more traditionally held view that migration between populations in *D. melanogaster* is extensive (Coyne *et al.* 1982; Coyne and Milstead 1987; Singh and Rhomberg 1987; Berry and Kreitman 1993).

On the basis of the test we developed to detect significant departures from symmetric migration, it would seem that asymmetrical gene flow between adjacent populations is relatively uncommon on the Australian continent. While it is true that the incidence of significant departures is likely to have been greater if the tests were based on more loci, the significant departures detected so far suggest that there is no consistent pattern in direction or location of asymmetry. Our results, therefore, are inconsistent with a systematic pattern of asymmetrical gene flow along the east coast of Australia and provide no support for the idea that the deviations from linearity in the body-size cline were caused by a regular influx of migrants from populations in warmer climates. Higher amounts of gene flow among low-latitude populations are also unlikely to have caused nonlinearity in the cline because isolation by distance was equally strong in the northern and southern halves of the cline.

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