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RELATIONSHIPS BETWEEN INFERRED LEVELS OF GENE FLOW AND GEOGRAPHIC DISTANCE IN A PHILOPATRIC CORAL, *BALANOPHYLLIA ELEGANS*

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Abstract.—When the dispersal capability of a species is considerably less than its geographic range, genetic differences between populations should increase with the distance separating those populations. This pattern should be most evident in linearly distributed species. The sessile solitary cup coral *Balanophyllia elegans* lives along nearly the entire Pacific coast of North America, yet its crawling larvae usually settle within 40 cm of their birthplace. In this paper, I document geographic patterns of allozyme differentiation within and among populations of *B. elegans* and estimate the proportion of observed geographic pattern attributable to gene flow between adjacent populations. Genetic subdivision among localities separated by up to 3000 km was high (F_{ST} = 0.283, SE = 0.038). Inferred gene flow between pairs of localities ($\hat{M}$, individuals per generation) correlated inversely with the geographic distance between those localities, consistent with the pattern expected for a species at equilibrium in which gene flow occurred exclusively between adjacent localities. Within localities, patches separated by 4 to 30 m were also significantly subdivided, but genetic differentiation between patches did not vary significantly with the distance separating them. Simulations revealed that the power to detect genetic pattern expected from gene flow between adjacent populations increased with both the number of loci used to infer gene flow and the heterozygosity of those loci. Simulations also verified that when geographic distance poorly approximated the number of steps between populations, reduced major-axis regression more accurately portrayed the structural relationship between gene flow and separation than did ordinary least-squares regression. Attenuation of gene flow with distance explained 15% of the between-locality pattern of genetic differentiation in *B. elegans*. The remaining variation appeared to be due to neither natural selection nor a recent rangewide recolonization. Loci from the northern sampled localities, however, had fewer alleles than those from the remainder of the range, suggesting these localities had been recolonized recently following Pleistocene cooling.

Key words.—Biogeography, geographic range, isolation by distance, larval dispersal, limited dispersal, Pleistocene, recolonization, RMA regression, speciation, stepping stone.

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Limited gene flow between populations promotes divergence via genetic drift and natural selection. The evolutionary consequences of restricted gene flow depend not only on the number of propagules moving between populations, but also on the geographic relationships between source and recipient populations. In particular, differentiation should increase when propagules move only between proximate populations. Wright (1943) first outlined this process, terming it "isolation by distance." Wright originally envisioned this process occurring within continuously distributed populations. A similar pattern of spatial differentiation arises between discrete populations when only immediately adjacent populations exchange genes (the "stepping stone" model; Kimura and Weiss 1964). Determining how populations differentiate in the absence of physical barriers to gene flow depends critically on whether gene flow proceeds largely between neighboring populations or, alternatively, whether populations exchange genes without geographic bias.

The relationships established among gene flow, geographic range, and genetic differentiation may determine both how speciation occurs and, ultimately, why clades possessing different dispersal capabilities may accumulate different numbers of species. Parapatric speciation, in which reproductive isolation evolves between populations maintaining a narrow zone of contact, does not require physical barriers to gene flow but does require gene flow to occur only between adjacent populations (Endler 1977). This mode of speciation may provide a mechanism for the genesis of species in the ocean, which generally lacks obvious physical barriers (Palumbi 1992). The rate of cladogenesis in benthic marine organisms with different types of devel-

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opment may also depend on the relationship between gene flow and geography (Hansen 1978; Jablonski 1986; Russell and Lindberg 1988). Hansen (1978) and Jablonski (1986) proposed that taxa with limited larval dispersal should exhibit greater rates of species turnover than related taxa with broadly dispersing larvae because of both relatively high extinction rates (stemming from reduced geographic range) and speciation rates (resulting from increased genetic subdivision).

Limited dispersal of larvae (in some cases less than 1 m) has been observed directly in many benthic marine taxa, including algae (Paine 1979), sponges (Bergquist et al. 1970), cnidarians (Ostarello 1976; Gerrodette 1981), molluscs (Janson 1983), crustaceans (Knowlton and Keller 1986), bryozoans (Keough and Chernoff 1987) and ascidians (Olson 1985; Grosberg 1987). Such philopatric dispersal should lead to increased genetic subdivision between populations and to greater speciation rates caused by decreased gene flow (Scheltema 1971; Hansen 1978; Stanley 1979; Jablonski 1986). Comparisons of sister taxa with different larval types support the expected inverse correlation between the magnitude of genetic differentiation among populations of benthic marine organisms and the dispersal potential of their larvae (Berger 1973; Janson 1987; McMillan et al. 1992; Hunt 1993; Duffy 1993).

Whether limited larval dispersal translates into a geographic pattern of gene flow consistent with a stepping-stone model remains unclear because mechanisms other than the movement of larvae may contribute to gene flow in marine invertebrates. Alternative mechanisms include the dispersal of gravid females (Highsmith 1985; Jokiel 1991) and of sperm (Grosberg 1991). In addition, the extinction and recolonization of populations brought on by transient environmental change could alter the expected geographic pattern of genetic differentiation (Slatkin 1993).

Quantitative assessment of whether genetic pattern conforms to a stepping-stone model requires expectations against which empirical data can be compared. Slatkin and Maddison (1989) and Slatkin (1993) recently developed models that generate such expectations and evaluated the statistical power of these methods using simulated genetic data. Slatkin (1993) showed that gene flow obeying the stepping-stone model leaves a characteristic pattern that can be detected using as few as ten allozyme loci. Furthermore, the power to detect such a pattern increases for populations distributed in one dimension rather than in two. These papers, however, do not address in detail how the number of loci surveyed, the amount of variation at these loci, and the geographic separation between sampled populations affect the power to detect pattern and how changes in these parameters may bias such tests.

Here, I document geographic patterns of differentiation of seven allozyme loci within and among populations of the coral *Balanophyllia elegans* Verrill. *Balanophyllia elegans* is a small (7–15 mm diameter) solitary scleractinian broadly distributed along the west coast of North America. Adults live attached to hard surfaces. Previous direct measurements of the dispersal of its crawling larvae suggest extremely limited movement ($\bar{x}$ = 39 ± 33 cm SD) (Gerrodette 1981). This philopatric larval dispersal and wide, nearly linear geographic rather closely approximates the assumptions of the stepping-stone model. As the mechanisms and events responsible for gene flow may vary with spatial scale, I characterized the pattern of genetic differentiation in *B. elegans* both on a large scale, between 18 subtidal localities spanning 3000 km, and on a smaller scale, between patches within these localities. I then compared the observed pattern of genetic differentiation with distance to expectations generated by a simulated stepping-stone model, using an equal number of similarly variable loci, to determine whether gene flow proceeded primarily between neighboring populations.

Materials and Methods

Natural History of Balanophyllia elegans

The geographic range of *Balanophyllia elegans* extends from Baja California (lat. 29°44′N) (Gerrodette 1979b) north into Alaska (to about 58°N) (Barr and Barr 1983; N. McDaniel pers. comm. 1990). *Balanophyllia elegans* corals live on rocky substrata from the intertidal occasionally down to a depth of 500 m (Durham and Barnard 1952). Densities generally peak at depths of 12–20 m depth and often decline considerably beyond 25 m (Gerrodette 1979b; pers. obs.). Local densities at depths of 10–15 m can exceed 500/m^2 (Fadlallah 1983; pers. obs.).

Although some other dendrophyllid corals brood asexual offspring (Ayre and Resing 1986), *B. elegans* reproduces strictly sexually. Females isolated in the laboratory do not release larvae after one reproductive season, whereas those paired with males do (M. Hellberg unpubl. data). Sex ratios in the field are equal (Fadlallah and

Pearse 1982). Both sexes reach maturity in about 2 yr. The average generation time is 3.5 yr and the maximum lifespan is about 10 yr (Fadlallah 1983). Males release sperm during a short period of time in the fall. The distance traveled by successful sperm of *B. elegans* is unknown. Studies on other benthic marine invertebrates suggest sperm may affect gene flow within, but not between, localities sampled in this study (Pennington 1985; Grosberg 1991).

Females brood planula larvae throughout much of the year, annually releasing 10–50 3-mm planulae primarily in late winter (Fadlallah and Pearse 1982; Fadlallah 1983; pers. obs.). These planulae crawl over hard substrata for one to several days before attaching, usually very close to the mother (Gerrodette 1981; Fadlallah 1983).

Collection of Samples

I collected samples from 18 localities spanning a distance of over 3000 km (fig. 1). Abbreviations and coordinates for sampled localities, in descending order of latitude, are as follows: British Columbia—HOY (Hoya Pass, Moresby Island, 52°41′N, 131°42′W), MCI (McInnes Island, Hecate Strait, 52°16′N, 128°44′W), BRS (Stubb's Island, Broughton Strait, 50°37′N, 126°49′W), NAN (Nanaimo, Dodd Narrows, 49°08′N, 123°49′W), and BAM (Bamfield, Grappler Inlet, 48°51′N, 125°08′W); Washington—TAT (Tatoosh Island, Landing Cove, 48°23′N, 124°45′W); Oregon—CAR (Cape Arago, Sunset Bay, 43°20′N, 124°23′W); California—TRN (Trinidad Harbor, 41°03′N, 124°08′W), CSP (Caspar, N of Point Cabrillo, 39;°21′N, 123°49′), BOD (off Bodega Bay, off Mussel Point, 38°21′N, 123°05′W), CRZ (Santa Cruz, Lighthouse Point, 36°56′N, 122°02′W), MON (Monterey, W of Coast Guard Jetty, 36°34′N, 121°52′), SIM (San Simeon, S of pier, 35°37′N, 121°08′), GOL (Goleta, Naples Reef, 34°26′N, 119°57′W), EAN (East Anacapa Island, Survey Rock, 34°01′N, 119°22′W), and PTL (Point Loma, 18m "Central" buoy of Dayton et al. [1992], 32°43′N, 117°16′W); Baja California—PTB (Punta Banda, off La Bufadora, 31°43′N, 116°45′W), and ISG (Isla San Geronimo, 29°45′N, 115°48′W). Other than the gap between Tatoosh Island, Washington and Cape Arago, Oregon, adjacent localities were ≤ 280 km apart (see table 5). At each locality, I gathered individuals from each of three to eight patches. Patches were separated by 4 to 6 m and were located within an area of 150 to 1000 m^2. I defined patches as all individuals found within a circle of radius 33 cm, the neighborhood distance calculated from direct measurements of larval dispersal (fig. 2 of Gerrodette 1981). I collected only adult corals (those greater than approximately 6 mm in diameter [Gerrodette 1979a] or about 150 mm^3 volume, Fadlallah 1983). All patches were located 5 to 20 m below mean lower low water, except Tatoosh Island (3–5 m) and Trinidad Head (2.5–4 m). I estimated the distance between patches within localities based on the number of body lengths between them.

All localities were sampled over the course of a single breeding season (April–October 1990), except Caspar (October 1989), Monterey (April 1991), and Bamfield (October 1991). I removed encrusting algae and epifauna from coral samples before freezing them in liquid nitrogen in the field. I also removed embryos found within females to avoid confounding larval and maternal genotypes. Samples were stored at −80°C.

Electrophoresis

To characterize individuals genetically, I chose to use allozymes rather than follow alternative approaches (e.g., mitochondrial DNA sequence data) for two reasons. First, mean confidence intervals for levels of gene flow estimated from molecular data will generally be no smaller than those obtained from allozymes because of technical limitations on the number of individuals and loci that can be assayed presently using molecular techniques (Slatkin and Maddison 1989). Second, the phylogenetic approaches used to estimate gene flow based on sequence data are unsuitable for detecting low levels of gene flow (Slatkin and Maddison 1989).

After an initial survey, I chose to assay seven highly polymorphic loci. I characterized seven-locus genotypes for individuals collected from all patches at each of the 18 locations shown in figure 1. I electrophoresed all samples using 12% (w/v) starch gels. Enzyme stains were modified from Selander et al. (1971). I assayed three loci—hexokinase (*Hk,* EC 2.7.1.1), phosphoglucomutase (*Pgm,* EC 2.7.5.1), and phosphoglucose isomerase (*Pgi,* EC 5.3.1.9)—using the pH 8.0 Tris-citrate buffer system of Selander et al. (1971). Four μl of 2-mercaptoethanol were added to 300-ml starch gels just before pouring to improve the resolution of bands for *Pgi.* Four other loci—triosephosphate isomerase (*Tpi,* EC 5.3.1.1), peptidase (*Pep,* EC 3.4.11/13.−), aspartate aminotransferase (*Aat,* EC 2.6.1.1), and leucine aminopeptidase (*Lap,* EC 3.4.11.1/2)—were assayed using the more dilute pH 8.0 Tris-citrate buffer

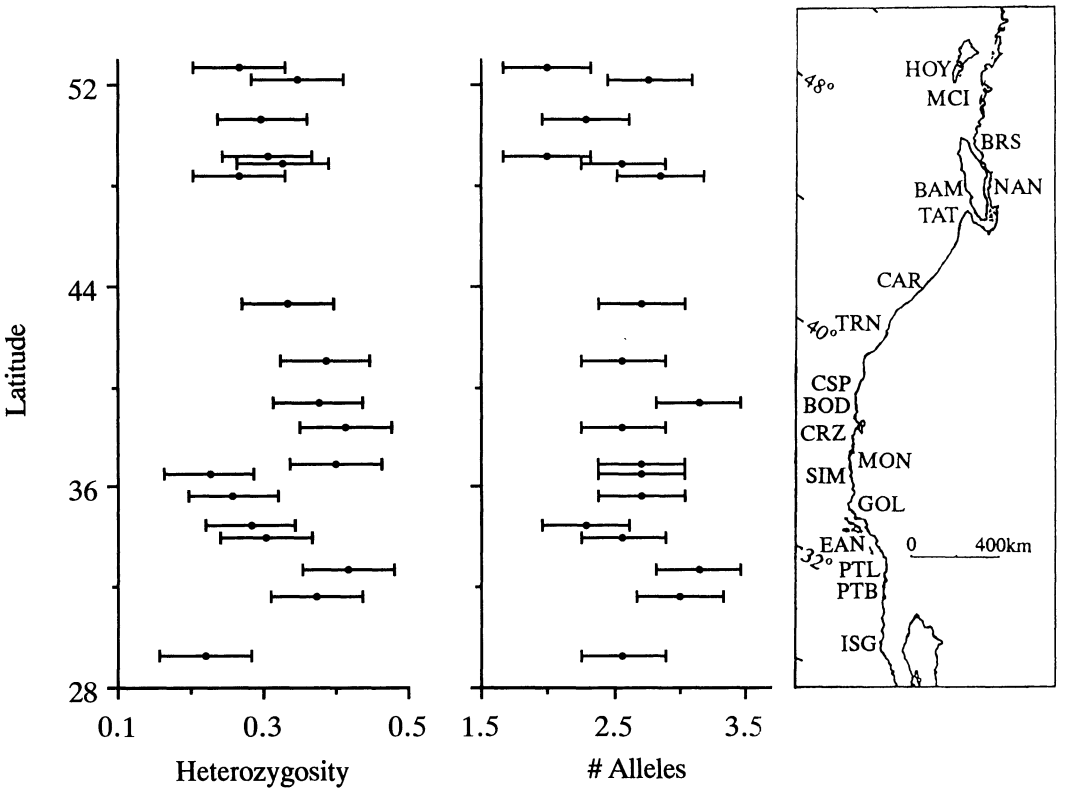

FIG. 1. Geographic variation in heterozygosity (left) and observed number of alleles per locus (right) within *Balanophyllia elegans* as determined from seven polymorphic loci (mean ± SE). See Materials and Methods for locality abbreviations.

system of Ward and Beardmore (1977). Leucyl alanine was used as a substrate for the peptidase stain.

Laboratory crosses confirmed that electromorphs were allelic (M. Hellberg unpubl. data). Alleles were numbered to indicate their percentage of mobility relative to the most common allele at that locus from the Santa Cruz population. Standards from Santa Cruz were run with individuals from each locality until all alleles were identified. Lineup gels were then used to confirm these identifications. Two null alleles (at *Pgi* and *Hk*) and one allele at the *Aat* locus that could not be consistently resolved were lumped with the most common allele at their respective loci. Two internal standards were run at two positions within each gel.

Analysis

I used Wright's *F*-statistics (Wright 1978) to characterize overall genetic subdivision. These statistics use departures from levels of heterozygosity expected under complete panmixia to partition total inbreeding (F_{IT}) into components resulting from inbreeding within subpopulations (F_{IS}) and subdivision among populations (F_{ST}). For selectively neutral loci, these estimates depend solely on the amount of gene flow among populations (assuming migration rates far exceeding mutation; Slatkin 1985), thus allowing estimates from different loci to be combined. Gene flow (Nm), the average number of migrants exchanged among populations each generation, can be estimated from this measure of genetic subdivision using the relationship $Nm = (1 - F_{ST})/4F_{ST}$. This provides an unbiased estimator of gene flow relatively insensitive to selection (Slatkin and Barton 1989).

Wright's parameters F_{IT}, F_{IS}, and F_{ST} were calculated using the estimators F, f, and θ of Weir and Cockerham (1984), respectively. I computed allele frequencies and hierarchical F statistics from genotype frequencies using the program of Weir (1990). This program was also used to calculate 95% confidence intervals about F statistics

by bootstrapping over loci. Standard errors of the mean were calculated by jackknifing over loci.

Two different spatial hierarchies were used to calculate the F statistics. For the first (large scale), I defined subpopulations as localities within the total range sampled. For the second (small scale), I defined subpopulations as patches within each locality (resulting in 18 different analyses).

To discern different geographic processes of gene flow from spatial patterns of differentiation, Slatkin and Maddison (1990) and Slatkin (1993) suggested using $\hat{M}$, the estimate of Nm calculated separately for pairs of populations. The simulations of Slatkin and Maddison (1990) demonstrated, and subsequent analytical work has confirmed (Slatkin 1991), that in a one-dimensional stepping-stone model the expected slope of a regression of $\log_{10} \hat{M}$ versus $\log_{10}$ distance of separation (in number of steps) is -1.0; that is, gene flow between localities inversely correlates with the number of steps separating populations. Under a two-dimensional stepping stone, the expected slope of the regression is approximately -0.5, (i.e., gene flow varies as the inverse square of the number of steps separating populations). I computed $\hat{M}$ (based on θ) for all pairwise combinations of populations using a program provided by M. Slatkin. Distance between localities was measured as the shortest nautical distance between localities reckoned from 1,000,000:1 scale maps.

The significance of the relationship between log distance and log $\hat{M}$ cannot be evaluated using standard regression techniques, as the regression is based on nonindependent, pairwise comparisons. Instead, I used Mantel's test to assess whether the correlation between the matrix of log distance of separation and the observed matrix of log $\hat{M}$ was significantly greater than correlations between log distance and randomized matrices of log $\hat{M}$. Randomized matrices were generated by shuffling rows and then columns (not cells) of the observed log $\hat{M}$ matrix (Manly 1991). I then standardized elements in the observed matrices to have a mean of zero and a variance of one (so the matrix of ordinary-least-squares [OLS] regression coefficient would equal the matrix correlation coefficient) and used the same randomization approach iteratively to establish 95% confidence intervals of the matrix regression correlation (Manly 1991). I used 5000 iterations in all cases. The resulting confidence intervals were identical to two decimal places to those for OLS regressions determined using degrees of freedom based on the number of populations sampled (not on the number of pairwise comparisons). Subsequent to this finding, I used OLS regression to determine whether the slope of the regression differed from zero.

Slatkin and Maddison (1990) and Slatkin (1993) used OLS regression to determine the slope of the relationship between gene flow and the number of steps separating populations. OLS regression assumes the independent variable (the number of steps separating populations, in this case) is measured without error. Although this assumption holds true for simulated data, it does not for actual data, where geographic distance approximates the number of steps between populations. Using OLS regression whenever error exists in the independent variable will underestimate the slope of the regression (McArdle 1988; LaBarbera 1989). In such instances, reduced-major-axis (RMA) regression should provide a better estimator of the relationship between the variables (McArdle 1988; LaBarbera 1989; see below). The slope of significant regressions was therefore determined using RMA regression. Asymmetric 95% confidence intervals about the slope of the RMA regressions were calculated following McArdle (1988), using degrees of freedom appropriate for the number of populations sampled.

To determine whether one locus contributed disproportionately to the geographic pattern of genetic differentiation, I excluded loci one at a time (jackknifed over loci) and recalculated the slope of log $\hat{M}$ versus log distance. I also excluded one locality at a time from the large-scale analysis to determine whether one locality disproportionately affected the slope.

If *B. elegans* had undergone a recent expansion of geographic range, levels of genetic variation in the recolonized areas should be reduced. To evaluate this possibility, I calculated expected heterozygosities ($H_e = 1 - \Sigma p_i^2$) at each locality using allele-frequency data for each locus and then averaging over the seven loci surveyed. Expected, rather than observed, heterozygosities were used so that comparisons between locations would not be obscured by inbreeding within locations. As an alternative measure of genetic variation, I also calculated the number of alleles per locus for each locality. Leberg (1992) showed experimentally that the number of alleles per locus may be a more sensitive indicator of past genetic bottlenecks than are multilocus measures of heterozygosity.

Simulations

I used a simulation model developed by Slatkin (1993) to determine how the power to detect a pattern consistent with the stepping-stone model varied with (1) the number of loci used, (2) the level of variation at those loci, and (3) the distance separating sampled populations. I also evaluated the relative proficiency of OLS regression and RMA regression to reveal the true relationship between gene flow and the number of steps separating sampled localities when there was error in the measurement of the number of steps between localities.

The simulations generated and analyzed allelic frequencies for diploid organisms found in populations of 10,000 individuals connected exclusively by gene flow between adjacent populations. Each replicate (= locus) of the simulation consisted of two parts. First, the history of each sampled gene was traced back to a single common ancestor. Generations were assumed to be discrete. Receding backward in time, each gene could, in the previous generation, (1) coalesce with another sampled gene from within its same population, (2) immigrate from another population, or (3) do neither of the first two. The respective probabilities of these events were determined by the pooled size of all populations and the pattern of gene flow between them. In all of my simulations, I sampled 25 diploid individuals from each of 11 populations. Mating within these populations was random. Sampled populations were centered in a one-dimensional array of 101 populations and separated from their adjacent sampled population by two steps. Each generation, a fraction, $1/m$, of the population consisted of immigrants drawn equally from the two adjacent populations.

Once the ancestry of the sampled genes had been established, the second part of the simulation assigned allelic identities to them. Starting with the common ancestor to all sampled genes, allelic identity changed independently along each branch of the phylogeny at a rate determined by mutation and the length of time passing between nodes. An infinite alleles model of mutation was assumed, so each mutation produced a unique allele.

A single iteration consisted of 7 to 20 such replicates. Resulting gene-frequency data were analyzed by regressing $\log_{10}(\hat{M})$ versus $\log_{10}$(distance of separation) as described above. The distribution of regression slopes from 100 iterations was compared to analytical expectations to establish the empirical power of different types of data to detect patterns established by gene flow between adjacent populations.

I assessed the relative performance of OLS and RMA regression when the distance of separating populations was measured with error by adding to the true number of steps between populations the product of this true value and a percentage of error, the absolute value of which varied randomly between zero and a specified maximum:

$$\text{distance with error} = (1 + \%\ \text{error}) \cdot (\text{true distance})$$

$$\text{where \% error} = \pm \text{ maximum \% error specified in figure 4.}$$

I used the resulting distance with error (measured number of steps) in subsequent regression analyses.

Results

The seven loci surveyed were highly polymorphic at most of the localities sampled (table 1). Within localities, patches showed substantial allozyme variation (see the Appendix). Subdivision between patches (θ) at this small spatial scale was significant within all localities in which samples were collected from eight patches (table 2). In contrast, inbreeding within localities (F) and patches (f) was significant at only a few localities. Each locus produced similar F statistics (table 3), other than large values of F and f at *Aat* primarily because of a heterozygote deficit within the CAR locality.

Differences in expected heterozygosities among localities (fig. 1) were not significant ($F = 0.784$, $P > 0.70$), although statistical tests for such differences have low power (Archie 1985). The average number of alleles per locus likewise showed no significant differences across localities ($F = 1.525$, $P > 0.10$). Contrasts of means suggested localities south of Santa Cruz were less heterozygous than others ($F = 4.23$, $P = 0.042$) and localities from Tatoosh north had fewer alleles per locus than did those from Cape Arago south ($F = 9.064$, $P = 0.0033$).

Large-scale hierarchical F statistics were calculated using localities as subpopulations. The values of both F and θ were large and differed by no more than a factor of 2.5 across loci (table 4). The coefficient of inbreeding within locations (f) was smaller and more variable across loci. All F statistics were significantly different from zero (table 4). The mean θ corresponded to an average

M (or Nm) between locations of 0.63, or a little more than one immigrant between populations every other generation. Gene flow between many localities was even lower (i.e., $\hat{M} < 0.5$) (fig. 2, table 5). Those localities showing relatively high levels of gene flow ($\hat{M} > 1.0$) were isolated by less than 1600 km.

The simulations of stepping-stone models by Slatkin and Maddison (1990) and Slatkin (1993) suggest the equilibrial slope of a log-log regression of $\hat{M}$ versus separation for populations distributed along a single dimension should be -1.0. For the large-scale analysis of *B. elegans,* the slope of the RMA regression was -0.73 (fig. 2). The asymmetric 95% confidence interval around this slope was bounded by -0.44 and -1.51. Randomization tests showed the OLS regression between log $\hat{M}$ and log distance of separation was significant (slope = -0.28, $P < 0.002$), and the 95% confidence limits for the matrix correlation coefficient were -0.40 and -0.16.

Slopes of the log-log RMA regression determined by excluding loci one at a time from the analysis were all significantly negative and slightly steeper than those based on the entire data set (table 6). Of the 153 pairwise values of θ, 6 were negative, and thus corresponding $\hat{M}$ values were undefined. Values reported in table 6 excluded these pairs. I also reanalyzed the slopes substituting the largest $\hat{M}$ for the subset of loci for the undefined values. This produced slightly more negative slopes, which reflected the same pattern as table 6. These similar patterns imply no single locus determined the overall observed negative correlation between log $\hat{M}$ and log separation. Jackknifing over locations likewise suggested that no single locality disproportionately affected the inverse structural relationship (SE = 0.024).

The y-intercept of the OLS regression of log $\hat{M}$ versus log distance was 0.502, which corresponds to an $\hat{M}$ of 3.18 and a θ (F_{ST}) of 0.073 at a distance of 1 km. This value of F_{ST} fell within the 95% confidence interval for inbreeding within localities (f) and approximately equaled the average F_{IT} for patches within localities (table 2).

Of the 13 localities at which 8 patches were sampled, the slope of log-log OLS regressions of $\hat{M}$ versus distance between rocks differed significantly from zero only within Monterey (results not shown). In that instance, the slope was positive. Hence, gene flow generally appeared unrelated to distance of separation at this level of analysis.

Results of the simulations suggested the ability

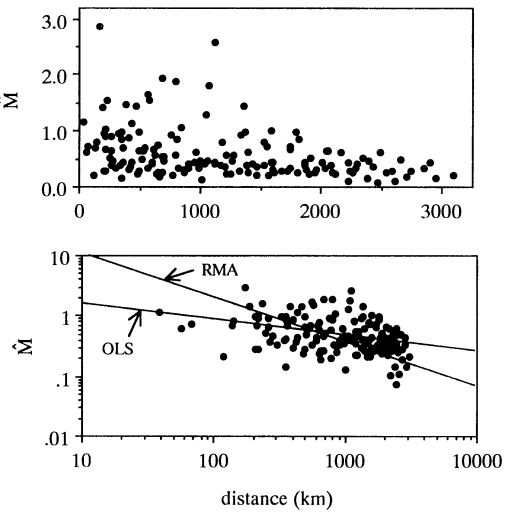

FIG. 2. Top. Inferred gene flow ($\hat{M}$, individuals/generation) versus geographic distance of separation (km) for all possible pairwise combinations of 18 populations of *Balanophyllia elegans.* $\hat{M}$ based on jackknifed average of F_{ST} over seven electrophoretically polymorphic enzyme loci as estimated by θ (Weir and Cockerham, 1984). Bottom. Ordinary-least-squares ($y = 0.502 - 0.280x$; $r^2 = 0.146$) and reduced-major-axis ($y = 1.833 - 0.733x$) regressions of log gene flow ($\hat{M}$) versus log distance of separation for all pairwise combinations of populations.

to detect the pattern produced by gene flow between adjacent populations depends not only on the number of loci surveyed, but also on the mutation rate (and thus the allelic diversity) of those loci (fig. 3). The three rates of mutation used ($\mu = 5 \times 10^{-7}$, 10^{-7}, and 5×10^{-8}) resulted in mean within-population heterozygosities ($\bar{x} =$ 0.28, 0.13, and 0.08, respectively) and numbers of alleles per locus ($\bar{x} =$ 2.15, 1.46, and 1.30, respectively), which spanned the range of variation typical in allozyme studies. The distribution of regression slopes from simulations based on more heterozygous loci were narrower than those determined using less variable loci, implying that the power to detect the genetic footprint of gene flow restricted to neighboring populations increases with the polymorphism of marker loci used to estimate levels of gene flow.

The slopes obtained by OLS and RMA regression techniques differed little when relatively large numbers of highly polymorphic loci were used and the number of steps separating localities was measured with either 0% (fig. 3e) or 25% error (fig. 4). With 50% error, the OLS regression underestimated the true slope, whereas the RMA regression was unbiased (fig. 4). At 99% error,

TABLE 1. Allele frequencies (in parentheses) for eighteen localities of *Balanophyllia elegans*. Alleles are numbered to indicate mobility relative to the most common allele from the Santa Cruz population. Localities are listed in order from north to south. See Material and Methods for locality abbreviations. N_i, number of individuals per locality. N_p, number of patches per locality.

	Locality								
Locus	HOY	MCI	BRS	NAN	BAM	TAT	CAR	TRN	CSP
N_i	128	128	72	126	128	128	60	128	68
N_p	8	8	6	8	8	8	6	8	4
HK	100 (0.133)	107 (0.008)	113 (0.014)	107 (0.063)	100 (0.094)	113 (0.035)	113 (0.533)	113 (0.332)	113 (0.066)
	93 (0.867)	100 (0.277)	100 (0.444)	100 (0.865)	93 (0.906)	107 (0.004)	100 (0.433)	107 (0.004)	107 (0.022)
		93 (0.715)	93 (0.542)	93 (0.072)		100 (0.703)	93 (0.033)	100 (0.664)	100 (0.177)
						93 (0.258)			93 (0.735)
PGM	100 (0.152)	100 (0.492)	100 (0.437)	100 (0.563)	100 (0.707)	100 (0.988)	112 (0.042)	100 (0.277)	100 (0.875)
	91 (0.848)	91 (0.508)	91 (0.563)	91 (0.437)	91 (0.293)	91 (0.012)	100 (0.958)	91 (0.723)	78 (0.125)
PGI	85 (1.00)	100 (0.004)	85 (1.00)	85 (1.00)	85 (0.984)	85 (1.00)	85 (1.00)	100 (0.098)	100 (0.140)
		85 (0.992)			74 (0.016)			85 (0.902)	85 (0.846)
		68 (0.004)							68 (0.015)
TPI	100 (0.777)	100 (0.859)	100 (0.590)	100 (0.452)	100 (0.918)	100 (0.656)	100 (0.675)	104 (0.238)	100 (0.617)
	93 (0.223)	93 (0.141)	93 (0.410)	93 (0.548)	93 (0.082)	93 (0.344)	93 (0.258)	100 (0.614)	93 (0.243)
							86 (0.067)	93 (0.148)	86 (0.140)
PEP	107 (0.039)	107 (0.035)	100 (0.979)	100 (1.00)	107 (0.398)	107 (0.008)	100 (0.542)	100 (0.352)	100 (0.676)
	100 (0.961)	100 (0.688)	92 (0.021)		100 (0.492)	100 (0.926)	92 (0.458)	92 (0.648)	92 (0.324)
		92 (0.277)			92 (0.110)	92 (0.066)			
AAT	92 (0.277)	92 (0.141)	100 (0.007)	100 (0.365)	100 (0.027)	100 (0.094)	100 (0.125)	82 (1.00)	100 (0.015)
	82 (0.723)	82 (0.836)	82 (0.007)	92 (0.631)	92 (0.773)	92 (0.066)	82 (0.867)		92 (0.007)
		53 (0.023)	53 (0.986)	82 (0.004)	82 (0.152)	82 (0.785)	53 (0.008)		82 (0.978)
					53 (0.047)	53 (0.055)			
LAP	111 (0.188)	111 (0.336)	111 (0.618)	111 (0.671)	111 (0.269)	111 (0.711)	111 (0.118)	111 (0.246)	111 (0.162)
	106 (0.609)	106 (0.641)	106 (0.264)	93 (0.329)	106 (0.590)	106 (0.215)	106 (0.658)	106 (0.246)	106 (0.353)
	100 (0.203)	93 (0.023)	93 (0.118)		100 (0.141)	100 (0.027)	100 (0.158)	100 (0.039)	100 (0.110)
						93 (0.047)	98 (0.058)	98 (0.453)	98 (0.368)
							96 (0.008)	96 (0.016)	96 (0.007)

TABLE 1. Extended.

Locus	Locality								
	BOD	CRZ	MON	SIM	GOL	EAN	PTL	PTB	ISG
N_i	128	128	128	128	128	128	128	48	46
N_p	8	8	8	8	8	8	8	3	4
HK	107 (0.008) 100 (0.848) 93 (0.144)	107 (0.004) 100 (0.910) 93 (0.086)	107 (0.004) 100 (0.949) 93 (0.047)	100 (0.887) 93 (0.113)	100 (0.402) 93 (0.598)	100 (0.703) 93 (0.293) 81 (0.004)	107 (0.012) 100 (0.688) 93 (0.297)	100 (0.969) 93 (0.031)	100 (0.891) 93 (0.109)
PGM	100 (0.449) 91 (0.551)	100 (0.672) 91 (0.328)	100 (0.164) 91 (0.824) 72 (0.012)	100 (0.250) 91 (0.746) 78 (0.004)	100 (0.062) 91 (0.938)	100 (0.238) 91 (0.762)	100 (0.477) 96 (0.047) 91 (0.477)	116 (0.031) 100 (0.542) 96 (0.073) 91 (0.219) 78 (0.135)	116 (0.076) 100 (0.794) 91 (0.130)
PGI	100 (0.316) 85 (0.684)	100 (0.793) 85 (0.207)	100 (0.152) 85 (0.844) 34 (0.004)	114 (0.016) 100 (0.168) 85 (0.691) 74 (0.125)	85 (1.00)	100 (0.141) 85 (0.859)	100 (0.422) 85 (0.578)	100 (0.187) 85 (0.813)	100 (0.152) 85 (0.848)
TPI	100 (0.789) 93 (0.211)	100 (0.984) 93 (0.016)	100 (0.828) 93 (0.172)	100 (1.00)	100 (1.00)	100 (1.00)	100 (0.992) 93 (0.008)	100 (1.00)	100 (1.00)
PEP	107 (0.234) 100 (0.426) 92 (0.340)	107 (0.012) 100 (0.531) 92 (0.457)	100 (0.996) 75 (0.004)	107 (0.012) 100 (0.984) 92 (0.004)	100 (0.887) 97 (0.113)	107 (0.004) 100 (0.914) 97 (0.008) 92 (0.074)	102 (0.070) 100 (0.848) 97 (0.027) 92 (0.055)	102 (0.114) 100 (0.761) 92 (0.114) 83 (0.011)	102 (0.022) 100 (0.924) 92 (0.054)
AAT	100 (0.008) 82 (0.992)	100 (0.379) 92 (0.309) 82 (0.312)	100 (0.004) 82 (0.996)	100 (0.141) 82 (0.859)	100 (0.570) 95 (0.008) 82 (0.422)	100 (0.172) 82 (0.828)	100 (0.406) 92 (0.008) 82 (0.586)	100 (0.167) 92 (0.010) 82 (0.823)	100 (0.022) 82 (0.978)
LAP	111 (0.379) 106 (0.090) 100 (0.141) 96 (0.391)	111 (0.276) 106 (0.355) 100 (0.367) 96 (0.004)	111 (0.324) 106 (0.508) 100 (0.109) 96 (0.059)	115 (0.012) 111 (0.692) 106 (0.188) 100 (0.109)	115 (0.004) 111 (0.289) 106 (0.438) 100 (0.199) 96 (0.070)	111 (0.137) 106 (0.492) 100 (0.273) 96 (0.098)	111 (0.422) 106 (0.417) 100 (0.133) 96 (0.051)	111 (0.291) 106 (0.417) 100 (0.198) 96 (0.167)	111 (0.011) 106 (0.522) 100 (0.413) 96 (0.022) 92 (0.033)

TABLE 2. Weir and Cockerham (1984) estimates of Wright's F statistics for patches within each locality. See Materials and Methods for locality abbreviations. Means and standard errors were obtained by jackknifing over loci. Mean F statistics whose 95% bootstrapped confidence intervals did not contain zero are marked with an asterisk. F = F_{IT} (individuals within localities), $\theta = F_{ST}$ (among patches within localities), f = F_{IS} (inbreeding within patches).

	F		θ		f	
Locality	Mean	SE	Mean	SE	Mean	SE
HOY	0.0039	0.0654	0.0285*	0.0125	−0.0257	0.0594
MCI	0.0779	0.0520	0.0516*	0.0174	0.0273	0.0427
BRS	0.0537	0.1047	0.0325*	0.0173	0.0217	0.1052
NAN	0.1135	0.0901	0.0620*	0.0296	0.0547	0.0886
BAM	0.0302	0.0340	0.0377*	0.0099	−0.0075	0.0417
TAT	0.0043	0.1000	0.0248*	0.0106	−0.0214	0.0983
CAR	0.0534	0.0894	0.0162*	0.0075	0.0373	0.0846
TRN	0.0482	0.0386	0.0191	0.0094	0.0299	0.0426
CSP	0.0308*	0.0169	−0.0064	0.0040	0.0369*	0.0163
BOD	−0.1152	0.0757	0.0171*	0.0083	−0.0576	0.0539
CRZ	0.1182	0.0424	0.0359*	0.0195	0.0852	0.0351
MON	-0.0290	0.0932	0.0544*	0.0415	−0.0902	0.0637
SIM	0.1044*	0.0326	0.0746*	0.0152	0.0330	0.0493
GOL	0.0943*	0.0688	0.0480*	0.0300	0.0478	0.0496
EAN	0.0093	0.0439	0.0150*	0.0117	−0.0058	0.0435
PTL	0.1020*	0.0410	0.0096*	0.0069	0.0934*	0.0412
PTB	0.0427	0.0568	0.0035	0.0060	0.0397	0.0611
ISG	0.0886	0.0733	0.0102	0.0149	0.0801	0.0822
Overall	0.0462	0.0138	0.0297	0.0051	0.0210	0.0114

both regressions models underestimated the actual slope, OLS regression more so than RMA (fig. 4). Varying the number of steps between sampled populations did not bias the observed slope of the regression (fig. 5).

DISCUSSION

Genetic differences between populations should increase with distance if gene flow occurs only between adjacent populations and gene flow and genetic drift have equilibrated (Slatkin 1993). This pattern should be reflected in an inverse relationship between $\hat{M}$ (the genetically inferred number of migrants moving between pairs of populations each generation) and the distance between populations. As expected on the basis of directly observed limited larval dispersal, populations of *Balanophyllia elegans* from localities spread over much of its broad geographic range were strongly genetically subdivided (table 4). The log of $\hat{M}$ between pairs of localities within *B. elegans* inversely correlated with the log of geographic separation (fig. 2), consistent with simulations in which gene flow passes solely between neighboring populations. However, spatial separation accounted for only about 15% of the variation in genetic differentiation over this range (based on r^2; see legend of fig. 2). The large proportion of geographic change in gene flow unaccounted for by the relationship between $\hat{M}$ and distance between populations indicates at least one of the following conditions: (1) the genetic

TABLE 3. Unweighted means (standard errors) over 18 localities of Weir and Cockerham (1984) estimates of Wright's F statistics for patches within localities calculated separately for each locus. F = F_{IT} (individuals within localities), $\theta = F_{ST}$ (among patches within localities), f = F_{IS} (inbreeding within patches).

Locus	F	θ	f
Hk	0.0686 (0.0269)	0.0294 (0.0099)	0.0592 (0.0237)
Pgm	0.0512 (0.0226)	0.0311 (0.0097)	0.0455 (0.0256)
Pgi	0.0321 (0.0321)	0.0177 (0.0153)	0.0133 (0.0324)
Tpi	0.0157 (0.0220)	0.0318 (0.0078)	−0.0184 (0.0256)
Pep	0.0293 (0.0235)	0.0150 (0.0054)	−0.0118 (0.0380)
Aat	0.1698 (0.0501)	0.0401 (0.0113)	0.1316 (0.0497)
Lap	0.0052 (0.0259)	0.0225 (0.0056)	−0.0174 (0.0251)

TABLE 4. Weir and Cockerham (1984) estimates of Wright's F statistics calculated separately for each locus for localities within the entire range. Means and standard errors were obtained by jackknifing over loci. Confidence interval obtained by bootstrapping over loci. F = F_{IT} (within total), $\theta = F_{ST}$ (among localities), f = F_{IS} (within localities).

Locus	F	θ	f
Hk	0.4508	0.3933	0.0945
Pgm	0.3535	0.3113	0.0614
Pgi	0.3805	0.3410	0.0599
Tpi	0.1988	0.2030	−0.0052
Pep	0.2854	0.2775	0.0109
Aat	0.4356	0.3375	0.1482
Lap	0.1801	0.1743	0.0071
Mean	0.3182	0.2832	0.0479
Standard error	0.0499	0.0379	0.0219
95% confidence interval	0.2408–0.4072	0.2248–0.3504	0.0180–0.0936

markers used to infer gene flow lack the power to resolve pattern; (2) the regression model used lacks power to resolve pattern; (3) the mating system of *B. elegans* violates assumptions of the stepping-stone model; (4) the width of the "linear" distribution is not negligible; (5) natural selection obscures the pattern; (6) populations have had insufficient time to come into equilibrium following recent colonization events; or (7) dispersal regularly occurs over a spatial scale greater than that sampled.

Power of Genetic Markers

The power to detect a pattern produced by simulated gene flow obeying a stepping-stone model increased with both the number of loci and the variability of those loci (fig. 3). For example, seven highly variable loci may offer greater power than twenty less variable loci (fig. 3c vs. fig. 3g). The power to detect pattern resulting from restricted gene flow will not increase indefinitely with the polymorphism of genetic markers used to infer gene flow (fig. 6 in Slatkin 1993). If a particular allele mutates before it spreads to adjacent populations, its distribution will provide no information on patterns of gene flow. The seven polymorphic allozyme loci I used to infer gene flow between populations were even more heterozygous than those produced in the simulations for $N\mu = 0.005$ (fig. 3); thus, the data reported for *B. elegans* should have been sufficiently powerful to detect pattern in a linear array of populations. Nevertheless, back mutations, or mutations to electrophoretically indistinguishable alleles, violate the infinite-alleles assumption of the simulations and may be one reason $\hat{M}$ remains relatively high even between some distant populations.

Power of the Regression Model

Ordinary-least-squares regression underestimates the slope of the relationship between gene flow and distance when the geographic distance between localities poorly approximates the number of steps genes take to traverse that distance (fig. 4). Barriers to dispersal in the form of sand (Burton et al. 1979) or currents (Tegner and Butler 1985; Ebert and Russell 1988) may lead to unequal gene flow between equally separated localities. In such instances, when geographic distance and the number of steps betwen localities cannot be reliably equated, figure 4 demonstrates that RMA regression provides a better estimate of the slope of the regression of log $\hat{M}$ and log distance (see also McArdle 1988; LaBarbera 1989). However, since RMA regression implies uncertainty in the both measured parameters, resulting regression lines should not be used to predict values of $\hat{M}$ for a given distance (Seim and Saether 1983). Thus, the large y-intercept of the RMA regression between log $\hat{M}$ and log distance has no biological meaning.

Distribution of Populations

Both theoretical analyses and simulations show that when gene flow takes place only between adjacent populations the slope of the regression between log $\hat{M}$ and log distance should be -1.0 for linearly distributed populations and -0.5 for populations spread in two dimensions (Slatkin and Maddison 1990; Slatkin 1991). Consequently, the power to detect genetic pattern produced by stepping-stone gene flow is greatest in species

TABLE 5. Pairwise estimates of gene flow between populations ($\hat{M}$) based on Weir and Cockerham's (1984) θ (individuals/generation, above diagonal) and shortest nautical distance between locations (km, below diagonal). See Materials and Methods for locality abbreviations.

	HOY	MCI	BRS	NAN	BAM	TAT	CAR	TRN	CSP	BOD	CRZ	MON	SIM	GOL	EAN	PTL	PTB	ISG
HOY	—	0.288	0.301	0.266	0.240	1.932	0.563	0.387	0.669	0.311	0.644	0.268	0.432	0.350	0.630	0.290	0.330	0.217
MCI	208	—	0.287	0.278	0.245	0.686	0.448	0.398	0.448	0.289	0.523	0.263	0.381	0.323	0.514	0.279	0.193	0.143
BRS	404	218	—	0.471	0.445	0.969	1.813	0.918	0.718	0.275	0.430	0.240	0.328	0.285	0.423	0.252	0.494	0.440
NAN	684	498	280	—	0.384	0.909	1.892	0.460	0.470	0.266	0.446	0.250	0.341	0.297	0.439	0.263	0.142	0.109
BAM	632	486	431	265	—	0.729	0.753	0.333	0.404	0.226	0.403	0.218	0.304	0.263	0.397	0.230	0.101	0.075
TAT	692	546	497	217	67	—	1.543	1.069	1.289	0.798	0.981	0.631	0.789	0.712	0.968	0.653	0.620	0.469
CAR	1270	1124	1075	795	645	578	—	0.645	1.440	0.435	0.572	0.341	0.446	0.395	0.564	0.356	1.006	0.858
TRN	1530	1384	1335	1055	905	838	260	—	0.679	0.395	0.633	0.337	0.470	0.405	0.622	0.355	0.296	0.218
CSP	1743	1597	1548	1268	1118	1051	473	213	—	0.687	0.947	0.429	0.645	0.536	0.926	0.458	2.583	1.433
BOD	1872	1726	1677	1397	1247	1180	602	342	137	—	1.419	1.035	0.852	1.650	0.674	0.308	0.344	0.226
CRZ	2060	1914	1865	1585	1435	1368	790	530	325	188	—	1.163	2.866	1.460	1.130	0.531	0.847	0.413
MON	2091	1945	1896	1616	1466	1399	821	561	356	219	39	—	0.811	0.981	0.866	0.268	0.208	0.132
SIM	2226	2080	2031	1751	1601	1534	956	696	491	354	172	143	—	0.943	0.909	0.491	0.574	0.252
GOL	2436	2290	2241	1961	1811	1744	1166	906	701	564	382	353	210	—	0.610	0.329	0.373	0.192
EAN	2490	2344	2295	2015	1865	1798	1220	960	755	618	436	407	264	57	—	0.522	0.693	0.402
PTL	2739	2593	2544	2264	2114	2047	1469	1209	1004	867	685	656	513	306	249	—	0.216	0.148
PTB	2857	2711	2662	2382	2232	2165	1587	1327	1122	985	803	774	631	424	367	118	—	1.559
ISG	3090	2944	2895	2615	2465	2398	1820	1560	1355	1218	1036	1007	864	657	600	351	233	—

TABLE 6. Intercepts and slopes of the RMA regression of $\log_{10} \hat{M}$ (individuals per generation) versus $\log_{10}$ nautical distance (km) based on data sets from which one locus had been excluded.

Locus excluded	Intercept	Slope
Hk	2.36	−0.919
Pgm	2.50	−0.985
Pgi	2.31	−0.882
Tpi	3.08	−1.180
Pep	2.26	−0.889
Aat	2.42	−0.944
Lap	2.20	−0.904
Mean	2.45	−0.958
Standard Error	0.296	0.1042

distributed in one dimension. Despite the bathymetric range of *B. elegans*, the steep North American continental shelf (depths >500 m within 30–45 km of shore) defines a linear distribution to the approximation of the 100 × 1 array of populations in the simulations. Population density of *B. elegans* peaks at only 12–20 m depth (Gerrodette 1979b), further strengthening this assumption.

Mating System

Demographically, the life history of *B. elegans* generally conforms to the assumptions of the model used to simulate gene flow between neighboring populations. Patches within localities were not significantly inbred (table 2). However, the significant subdivision between patches within localities (table 2) implies genes moving between localities will take longer to coalesce than predicted by the simulation model, which assumes panmixia within localities. This should reduce the observed $\hat{M}$ between localities below those expected from simulations. However, as subdivision between patches did not vary markedly across localities (see table 2), it should not bias the relationship between $\hat{M}$ and distance. Violation of the assumption of discrete generations should likewise reduce $\hat{M}$, but again should not alter the relationship as long as all localities are effected equally.

Natural Selection

Natural selection may underlie latitudinal variation observed at some allozyme loci in other benthic marine invertebrates (Corbin 1977; Koehn et al. 1980; Hoffman 1981; Hedgecock 1986). My genetic analysis of the population structure of *B. elegans* combined information

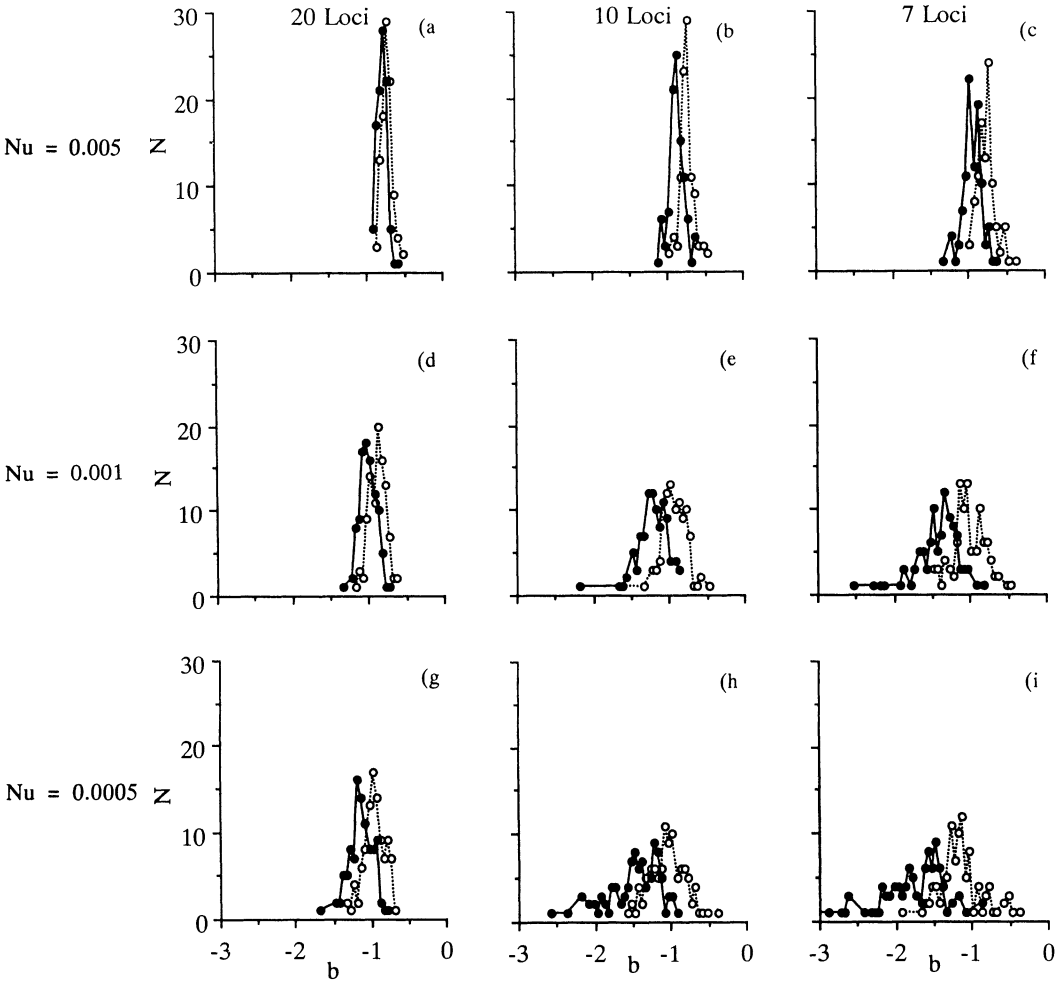

FIG. 3. Distributions of slopes (b) of ordinary-least-squares (open points) and reduced-major-axis (filled points) regressions of $\log_{10} \hat{M}$ (individuals/generation) versus $\log_{10}$(number of steps separating populations) in a one-dimensional stepping-stone model as a function of changes in the number of loci sampled and the product of population size and mutation rate ($N\mu$) of those loci. Samples of 25 diploid individuals were taken from each of 11 central populations, spaced two steps from the nearest sampled population, within a 101 × 1 array of populations. Histograms show the distribution of b for 100 simulated data sets. $N = 10{,}000$ for all populations. $Nm = 1.0$.

from seven loci, all of which produced similar estimates of subdivision (tables 3, 4) and change of $\hat{M}$ with distance (table 5). Provided these loci are not tightly linked, selection would be highly unlikely to create similar patterns in each. Nevertheless, the possibility remains that balancing selection at these loci homogenizes allele frequencies at different localities, thereby reducing genetic differentiation between localities and increasing corresponding estimates of gene flow (Karl and Avise 1992).

Equilibrium between Gene Flow and Genetic Drift

Temporal fluctuations in the magnitude of gene flow between pairs of populations weaken the correlation between $\hat{M}$ and geographic distance because gene flow and genetic drift require time to equilibrate (Slatkin 1993). Estimates of gene flow based on F_{ST} approach their equilibrium values over a time scale (in number of generations) close to the inverse of the migration rate, assuming mutation is small relative to migration,

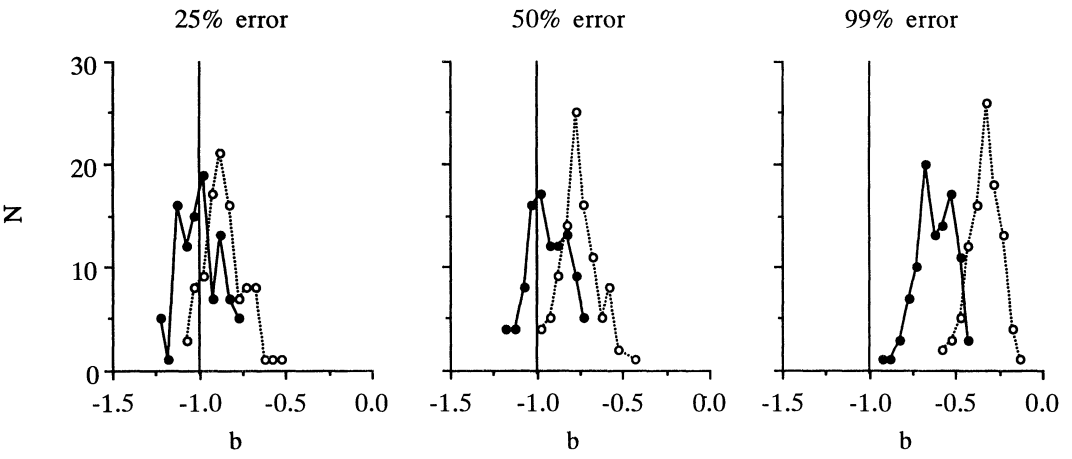

FIG. 4. Distributions of ordinary-least-squares (open points) and reduced-major-axis (filled points) regressions of $\log_{10} \hat{M}$ (individuals/generation) versus $\log_{10}$(number of steps separating populations) in a one-dimensional stepping-stone model showing the effects of measuring the number of steps between populations with error. Simulations were identical to those depicted in figure 3, except $N\mu = 0.001$ and 10 loci were sampled for each run.

and the inverse of the effective population size is far less than one (Crow and Aoki 1984). In *B. elegans,* very low migration rates imply that this approach to equilibrium could take many years. For gene flow between localities, using an estimate of $Nm = 1$, $N = 62{,}500$ (based on a sampled locality area of 250 m^2 and density of 250/m^2), a generation time of 3 yr, and Crow and Aoki's approximation [$t_{1/2} = \ln 2/(2m + 1/N)$], it would take 43,320 yr to go half way to equilibrium following a change in the amount of gene flow between populations. In contrast, equilibration between patches should occur much more rapidly. For gene flow between patches using $N = 350$ (based on a neighborhood area of 1.4 m^2 calculated from larval dispersal distances of Gerrodette [1981]) and $Nm = 5$, $t_{1/2} = 66.2$ yr.

Fossil remains suggest that *B. elegans* spanned much of its present geographic range during high sea-level stands 80,000 (from Bandon, Oregon [Zullo 1969] to Punta Banda, Baja California [Rockwell et al. 1989]) and 125,000 yr B. P. (Cayucos, California [G. L. Kennedy pers. comm. 1992] to Punta Banda [Rockwell et al. 1989]). Based on the preceding calculations, this period of time suffices for gene flow and drift to reach equilibrium. Furthermore, the weak correlation between log $\hat{M}$ and log distance stems primarily from low levels of gene flow between some proximate pairs of populations, and not from exceptionally high values between distant pairs of populations (see fig. 2). The opposite pattern would be expected following a recent range expansion (Slatkin 1993), which would result in transiently high levels of inferred gene flow between distant populations. The positive correlation between gene flow and distance between patches within the Monterey locality may reflect such an expansion, as some samples were taken from recently (<66 yr) introduced substrate at this locality.

Pleistocene cooling events latitudinally shifted the geographic ranges of a number of temperate Eastern Pacific shallow-water marine organisms (Addicott and Greene 1974; Rockwell et al. 1989). If Pleistocene cooling shifted the northern range limit of *B. elegans,* gene flow and drift would not be expected to be at equilibrium within this region. Although neither heterozygosity nor the number of alleles per locus (fig. 1) exhibited significant latitudinal variation over all populations, the number of alleles per locus was significantly lower for populations from Tatoosh Island north than for those from Cape Arago southward. This latitudinal division corresponds roughly to the geographic extent of recent (approximately 18,000 yr B. P.) glaciers (Easterbrook 1969; CLIMAP 1976). Relatively low levels of heterozygosity have been reported for other populations that have rapidly expanded following recent climatic changes (Highton and Webster 1976; table 2 in Reeb and Avise 1990). Fluctuations in sea level may similarly prevent the marine benthos on shallow continental shelves from reaching genetic equilibrium (Potts 1983), although these fluctuations probably have little

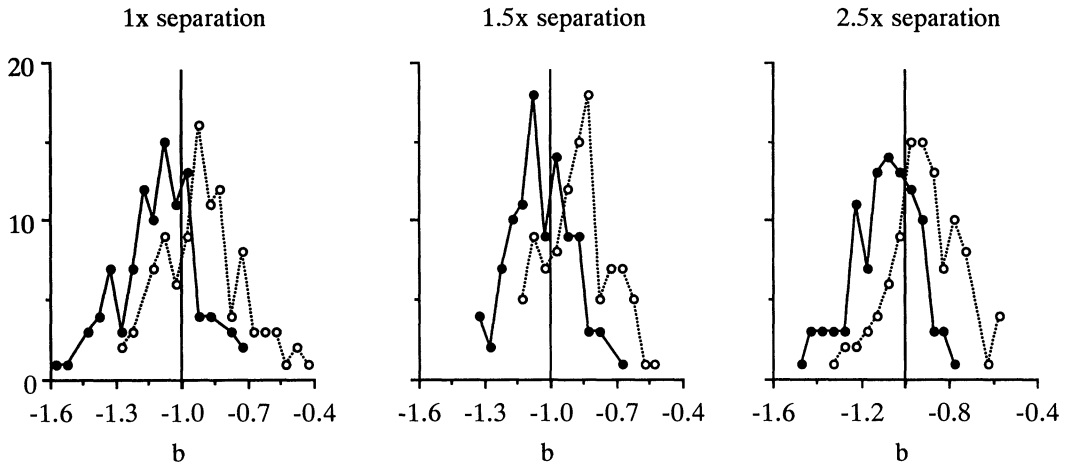

FIG. 5. Distributions of ordinary-least-squares (open points) and reduced-major-axis (filled points) regressions of $\log_{10} \hat{M}$ (individuals/generation) versus $\log_{10}$(number of steps separating populations) in a one-dimensional stepping-stone model showing the effect of varying the number of steps between sampled populations. Samples of 25 diploid individuals were taken from each of 11 central populations, spaced two ($1x$), three ($1.5x$), or four ($2x$) steps apart from the nearest sampled population, within a 101 × 1 array of populations. Histograms show the distribution of b for 100 simulated data sets. $N\mu = 0.001$, $Nm = 5.0$, and 10 loci were sampled for each run.

direct effect along narrow linear shelves such as the west coast of North America, as long as appropriate habitat (rocky substrate in the case of *B. elegans*) remains available when seas rise and fall (Valentine and Jablonski 1983).

Long-Distance Dispersal

Field experiments suggest *B. elegans* rarely disperses over large spatial scales. Gerrodette (1981) found no individuals on a large (approximately 15 m × 70 m) artificial reef that had been submerged 16 yr previously and was only 4 km from the nearest source population. Recent surveys of this and other artificial reefs in southern California submerged for from 6 to 22 yr (R.P. Lewis, Calif. Fish & Game, pers. comm.) documented no *B. elegans*, despite the occasional presence of another scleractinian (*Astrangia lajollaensis*) with habitat requirements similar to those of *B. elegans* (Chadwick 1991), but possessing planktonic larvae (Fadlallah 1982). Groups of *B. elegans* adults attached to small boulders or the shells of abalone (*Haliotis* sp.) might be rafted long distances by large kelp. Alternative mechanisms of gene flow over long distances seem less likely. Most individually detached adults (free of any substratum) do not survive simulated dispersal of over 16 km (M. Hellberg unpubl. data). Larvae released in laboratory rearings have never been observed to swim (Gerrodette 1981; Fadlallah and Pearse 1982; M. Hellberg pers. obs.).

The physical environment should determine levels of gene flow affected by passive means of dispersal, such as the rafting of adults. Thus, marine organisms with dispersal capabilities and geographic distributions similar to those of *B. elegans* should show similar patterns of genetic subdivision. Indeed, the average F_{ST} for *B. elegans* is nearly identical to those of other benthic marine invertebrates found on rocky substrate from the Pacific Northwest with crawling offspring (e.g., the gastropod *Nucella lamellosa*, Grant and Utter 1988; the octocoral *Alcyonium* sp., C. S. McFadden pers. comm. 1994; the anemone *Epiactis prolifera*, S. Edmands pers. comm. 1994). In contrast, over a comparable range, sessile marine invertebrates whose larvae develop in the plankton show much less genetic subdivision (Levinton and Suchanek 1978; Smith and Potts 1987; Palumbi and Wilson 1990).

Conclusions

The relationship between gene flow ($\hat{M}$) and distance in *Balanophyllia elegans* agreed with predictions of a simulated stepping-stone model at a large spatial scale (>3000 km), although distance of separation accounted for only 15% of geographic change in $\hat{M}$. Patches within localities separated by 3–50 m showed no pattern of change in gene flow with distance, despite significant subdivision between patches. Two alternative processes likely determine these ob-

served patterns: (1) insufficient time for gene flow and genetic drift to equilibrate over large spatial scales and (2) ongoing long-distance dispersal. These two processes predict that distance will account for different proportions of the variation in $\hat{M}$ at intermediate spatial scales. If distant populations have yet to come into equilibrium following historical changes in gene flow, then the magnitude of the slope of the regression between log $\hat{M}$ and log distance should increase at spatial scales somewhat larger than those between patches but eventually begin to decline at a spatial scale sufficiently large to prevent gene flow from equilibrating with genetic drift. Distance should explain much of the variability in $\hat{M}$ at this intermediate maximum. Alternatively, gene flow between distant localities may continually disrupt the approach of $\hat{M}$ to near-zero equilibrium values. Under this scenario, most localities exchange no genes during a given generation; thus, for most pairs of populations, $\hat{M}$ decreases each generation. Occasionally, however, gene flow between populations (sometimes distant, although more often relatively close) interrupts this approach to equilibrium, transiently increasing $\hat{M}$. The predicted relationship between $\hat{M}$ and distance under this scenario should mirror that at larger spatial scale, as the same mechanisms form pattern at each. Thus, distance should explain a similarly low proportion of the variation in $\hat{M}$ at intermediate spatial scales.

At the macroevolutionary level, resolving the mechanistic basis of the relationship between gene flow and distance in *B. elegans* would help circumscribe the possibility of parapatric speciation in marine organisms with similar biogeographic distributions and dispersal potential. For example, the gastropod *Nucella emarginata,* once considered a single species, actually consists of at least two sibling species (Palmer et al. 1990). If the relationship between $\hat{M}$ and distance in *B. elegans* proves strong at intermediate spatial scales, this would suggest the two species of *N. emarginata* could have become reproductively isolated while maintaining a narrow margin of sympatry. If, however, distance fails to account for much of the variability in gene flow at any spatial scale, then mechanisms other than parapatric speciation would be favored. The relative paucity of alleles in *B. elegans* from northern localities suggests that rapid latitudinal range shifts may often isolate small peripheral populations over geologic time. A comparative study on the geographic patterns of genetic differentiation in a similarly distributed organism possessing planktonic larvae should reveal whether clades with different modes of larval dispersal differ in their potential for parapatric speciation.

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Note Added in Proof

The program used to calculate pairwise values of $\hat{M}$ in this paper was in error. The slope of the OLS regression should be log $\hat{M}$ = 0.37 − 0.17 (log (distance)), with 95% confidence intervals extending from −0.272 to −0.083. The RMA regression shoud be log $\hat{M}$ = 1.62 − 0.60 (log (distance)), with assymetrical 95% confidence limits at −0.37 and −0.97. Log distance explained 8.6% of the variation in log $\hat{M}$. Thus, the regression slope was weaker than expected for a one-dimensional stepping stone at equilibrium, suggesting a greater role for either long-distance dispersal or historical changes in gene flow (discussed above) in shaping the relationship between gene flow and distance at large spatial scales. Relationships between gene flow and distance within localities remain insignificant. Results of simulations remain unchanged.

Appendix.

Allele frequencies (in parentheses) for *Balanophyllia elegans* from patches within eighteen localities. Alleles are numbered to indicate mobility relative to the most common allele from the Santa Cruz population. See Materials and Methods for locality abbreviations.

Site	Patch	*N*	HK	PGM	PGI	TPI	PEP	AAT	LAP
HOY	1	16	100 (0.219)	100 (0.156)	85 (1.00)	100 (0.875)	107 (0.062)	92 (0.344)	111 (0.156)
			93 (0.781)	91 (0.844)		93 (0.125)	100 (0.938)	82 (0.656)	106 (0.719)
									100 (0.125)
	2	16	100 (0.094)	100 (0.281)	85 (1.00)	100 (0.531)	100 (1.00)	92 (0.219)	111 (0.156)
			93 (0.906)	91 (0.719)		93 (0.469)		82 (0.781)	106 (0.750)
									100 (0.094)
	3	16	100 (0.062)	100 (0.062)	85 (1.00)	100 (0.750)	107 (0.125)	92 (0.344)	111 (0.219)
			93 (0.938)	91 (0.938)		93 (0.250)	100 (0.875)	82 (0.656)	106 (0.625)
									100 (0.156)
	4	16	100 (0.125)	100 (0.187)	85 (1.00)	100 (0.719)	107 (0.031)	92 (0.344)	111 (0.125)
			93 (0.875)	91 (0.813)		93 (0.281)	100 (0.969)	82 (0.656)	106 (0.656)
									100 (0.219)
	5	16	100 (0.281)	100 (0.156)	85 (1.00)	100 (0.875)	100 (1.00)	92 (0.156)	111 (0.312)
			93 (0.719)	91 (0.844)		93 (0.125)		82 (0.844)	106 (0.594)
									100 (0.094)
	6	16	100 (0.062)	100 (0.094)	85 (1.00)	100 (0.812)	107 (0.062)	92 (0.281)	111 (0.031)
			93 (0.938)	91 (0.906)		93 (0.188)	100 (0.938)	82 (0.719)	106 (0.719)
									100 (0.250)
	7	16	100 (0.062)	100 (0.188)	85 (1.00)	100 (0.875)	100 (1.00)	92 (0.250)	111 (0.125)
			93 (0.938)	91 (0.812)		93 (0.125)		82 (0.750)	106 (0.438)
									100 (0.438)
	8	16	100 (0.156)	100 (0.094)	85 (1.00)	100 (0.781)	107 (0.031)	92 (0.281)	111 (0.375)
			93 (0.844)	91 (0.906)		93 (0.219)	100 (0.969)	82 (0.719)	106 (0.375)
									100 (0.250)
MCI	1	16	100 (0.094)	100 (0.219)	85 (1.00)	100 (0.969)	107 (0.062)	92 (0.437)	111 (0.219)
			93 (0.906)	91 (0.781)		93 (0.031)	100 (0.531)	82 (0.563)	106 (0.781)
							92 (0.406)		
	2	16	100 (0.188)	100 (0.688)	85 (1.00)	100 (0.750)	107 (0.031)	92 (0.250)	111 (0.281)
			93 (0.812)	91 (0.312)		93 (0.250)	100 (0.719)	82 (0.719)	106 (0.688)
							92 (0.250)	53 (0.031)	93 (0.031)
	3	16	100 (0.188)	100 (0.625)	85 (1.00)	100 (0.906)	100 (0.750)	92 (0.062)	111 (0.156)
			93 (0.812)	91 (0.375)		93 (0.094)	92 (0.250)	82 (0.938)	106 (0.844)
	4	16	107 (0.062)	100 (0.469)	100 (0.031)	100 (0.844)	107 (0.062)	92 (0.031)	111 (0.281)
			100 (0.313)	91 (0.531)	85 (0.969)	93 (0.156)	100 (0.750)	82 (0.906)	106 (0.688)
			93 (0.625)				92 (0.188)	53 (0.062)	93 (0.031)
	5	16	100 (0.219)	100 (0.438)	85 (1.00)	100 (0.938)	100 (0.625)	92 (0.031)	111 (0.375)
			93 (0.781)	91 (0.562)		93 (0.062)	92 (0.375)	82 (0.969)	106 (0.625)
	6	16	100 (0.281)	100 (0.469)	85 (1.00)	100 (0.906)	107 (0.031)	92 (0.156)	111 (0.531)
			93 (0.719)	91 (0.531)		93 (0.094)	100 (0.750)	82 (0.844)	106 (0.469)
							92 (0.219)		
	7	16	100 (0.656)	100 (0.500)	85 (1.00)	100 (0.750)	100 (0.719)	92 (0.094)	111 (0.469)
			93 (0.344)	91 (0.500)		93 (0.250)	92 (0.281)	82 (0.875)	106 (0.500)
								53 (0.031)	93 (0.031)

APPENDIX. Continued.

Site	Patch	*N*	HK	PGM	PGI	TPI	PEP	AAT	LAP
	8	16	100 (0.281) 93 (0.719)	100 (0.531) 91 (0.469)	85 (0.969) 68 (0.031)	100 (0.812) 93 (0.188)	107 (0.094) 100 (0.656) 92 (0.250)	92 (0.062) 82 (0.875) 53 (0.062)	111 (0.375) 106 (0.531) 93 (0.094)
BRS	1	13	100 (0.577) 93 (0.423)	100 (0.615) 91 (0.385)	85 (1.00)	100 (0.423) 93 (0.577)	100 (1.00)	82 (1.00)	111 (0.577) 106 (0.231) 93 (0.192)
	2	11	113 (0.045) 100 (0.454) 93 (0.500)	100 (0.409) 91 (0.591)	85 (1.00)	100 (0.636) 93 (0.364)	100 (1.00)	82 (1.00)	111 (0.455) 106 (0.318) 93 (0.227)
	3	8	100 (0.563) 93 (0.437)	100 (0.125) 91 (0.875)	85 (1.00)	100 (0.500) 93 (0.500)	100 (1.00)	82 (1.00)	111 (0.687) 106 (0.250) 93 (0.063)
	4	4	100 (0.125) 93 (0.875)	100 (0.375) 91 (0.625)	85 (1.00)	100 (0.750) 93 (0.250)	100 (1.00)	92 (0.125) 82 (0.875)	111 (0.500) 106 (0.500)
	5	18	113 (0.028) 100 (0.444) 93 (0.528)	100 (0.583) 91 (0.417)	85 (1.00)	100 (0.528) 93 (0.472)	100 (0.917) 92 (0.083)	82 (1.00)	111 (0.667) 106 (0.194) 93 (0.139)
	6	18	100 (0.361) 93 (0.639)	100 (0.333) 91 (0.667)	85 (1.00)	100 (0.750) 93 (0.250)	100 (1.00)	100 (0.028) 82 (0.972)	111 (0.694) 106 (0.278) 93 (0.028)
NAN	1	16	107 (0.031) 100 (0.969)	100 (0.594) 91 (0.406)	85 (1.00)	100 (0.656) 93 (0.344)	100 (1.00)	100 (0.562) 82 (0.438)	111 (0.687) 93 (0.313)
	2	15	107 (0.133) 100 (0.800) 93 (0.067)	100 (0.633) 91 (0.367)	85 (1.00)	100 (0.167) 93 (0.833)	100 (1.00)	100 (0.300) 82 (0.700)	111 (0.833) 93 (0.167)
	3	16	107 (0.031) 100 (0.812) 93 (0.156)	100 (0.500) 91 (0.500)	85 (1.00)	100 (0.406) 93 (0.594)	100 (1.00)	100 (0.500) 82 (0.500)	111 (0.656) 93 (0.344)
	4	16	100 (0.781) 93 (0.219)	100 (0.469) 91 (0.531)	85 (1.00)	100 (0.250) 93 (0.750)	100 (1.00)	100 (0.594) 82 (0.406)	111 (0.469) 93 (0.531)
	5	16	100 (1.00)	100 (0.688) 91 (0.312)	85 (1.00)	100 (0.656) 93 (0.344)	100 (1.00)	100 (0.469) 82 (0.500) 53 (0.031)	111 (0.750) 93 (0.250)
	6	15	107 (0.133) 100 (0.867)	100 (0.500) 91 (0.500)	85 (1.00)	100 (0.567) 93 (0.433)	100 (1.00)	100 (0.033) 82 (0.967)	111 (0.600) 93 (0.400)
	7	16	107 (0.031) 100 (0.937) 93 (0.031)	100 (0.469) 91 (0.531)	85 (1.00)	100 (0.438) 93 (0.562)	100 (1.00)	100 (0.250) 82 (0.750)	111 (0.656) 93 (0.344)
	8	16	107 (0.156) 100 (0.750) 93 (0.094)	100 (0.656) 91 (0.344)	85 (1.00)	100 (0.469) 93 (0.531)	100 (1.00)	100 (0.188) 82 (0.812)	111 (0.719) 93 (0.281)
BAM	1	16	100 (0.182) 93 (0.812)	100 (0.719) 91 (0.281)	85 (1.00)	100 (1.00)	107 (0.562) 100 (0.375) 92 (0.062)	100 (0.031) 92 (0.906) 82 (0.063)	111 (0.250) 106 (0.687) 100 (0.063)
	2	16	100 (0.188) 93 (0.812)	100 (0.719) 91 (0.281)	85 (1.00)	100 (0.844) 93 (0.156)	107 (0.469) 100 (0.437) 92 (0.094)	92 (0.875) 82 (0.062) 53 (0.062)	111 (0.406) 106 (0.250) 100 (0.344)
	3	16	100 (0.031) 93 (0.969)	100 (0.750) 91 (0.250)	85 (1.00)	100 (0.906) 93 (0.094)	107 (0.469) 100 (0.500) 92 (0.031)	92 (0.750) 82 (0.188) 53 (0.062)	111 (0.156) 106 (0.625) 100 (0.219)
	4	16	100 (0.156) 93 (0.844)	100 (0.750) 91 (0.250)	85 (1.00)	100 (1.00)	107 (0.344) 100 (0.500) 92 (0.156)	100 (0.156) 92 (0.594) 82 (0.098) 53 (0.156)	111 (0.250) 106 (0.750)
	5	16	93 (1.00)	100 (0.656) 91 (0.344)	85 (0.969) 74 (0.031)	100 (1.00)	107 (0.219) 100 (0.750) 92 (0.031)	92 (0.625) 82 (0.313) 53 (0.062)	111 (0.219) 106 (0.781)
	6	16	93 (1.00)	100 (0.469) 91 (0.531)	85 (0.969) 74 (0.031)	100 (0.781) 93 (0.219)	107 (0.313) 100 (0.562) 92 (0.125)	100 (0.031) 92 (0.844) 82 (0.125)	111 (0.375) 106 (0.469) 100 (0.156)

APPENDIX. Continued.

Site	Patch	*N*	HK	PGM	PGI	TPI	PEP	AAT	LAP
	7	16	100 (0.125) 93 (0.875)	100 (0.875) 91 (0.125)	85 (0.969) 74 (0.031)	100 (0.938) 93 (0.062)	107 (0.406) 100 (0.406) 92 (0.188)	92 (0.813) 82 (0.156) 53 (0.031)	111 (0.281) 106 (0.594) 100 (0.125)
	8	16	100 (0.062) 93 (0.938)	100 (0.781) 91 (0.219)	85 (0.969) 74 (0.031)	100 (0.875) 93 (0.125)	107 (0.406) 100 (0.406) 92 (0.188)	92 (0.781) 82 (0.219)	111 (0.219) 106 (0.562) 100 (0.219)
TAT	1	16	113 (0.156) 100 (0.531) 93 (0.313)	100 (1.00)	85 (1.00)	100 (0.781) 93 (0.219)	100 (0.969) 92 (0.031)	100 (0.062) 82 (0.781) 53 (0.156)	111 (0.719) 106 (0.125) 93 (0.156)
	2	16	113 (0.031) 100 (0.687) 93 (0.281)	100 (0.969) 91 (0.031)	85 (1.00)	100 (0.625) 93 (0.375)	100 (0.969) 92 (0.031)	100 (0.125) 82 (0.781) 53 (0.094)	111 (0.656) 106 (0.188) 100 (0.062) 93 (0.094)
	3	16	113 (0.031) 100 (0.594) 93 (0.375)	100 (1.00)	85 (1.00)	100 (0.531) 93 (0.469)	100 (0.844) 92 (0.156)	92 (0.062) 82 (0.906) 53 (0.031)	111 (0.688) 106 (0.281) 100 (0.031)
	4	16	107 (0.031) 100 (0.750) 93 (0.219)	100 (1.00)	85 (1.00)	100 (0.656) 93 (0.344)	100 (1.00)	100 (0.125) 92 (0.125) 82 (0.719) 53 (0.031)	111 (0.719) 106 (0.187) 100 (0.062) 93 (0.031)
	5	16	113 (0.031) 100 (0.844) 93 (0.125)	100 (0.938) 91 (0.062)	85 (1.00)	100 (0.469) 93 (0.531)	107 (0.031) 100 (0.969)	100 (0.031) 82 (0.937) 53 (0.031)	111 (0.719) 106 (0.219) 100 (0.031) 93 (0.031)
	6	16	100 (0.750) 93 (0.250)	100 (1.00)	85 (1.00)	100 (0.719) 93 (0.281)	107 (0.031) 100 (0.937) 92 (0.031)	100 (0.219) 92 (0.031) 82 (0.750)	111 (0.781) 106 (0.156) 100 (0.031) 93 (0.031)
	7	16	113 (0.031) 100 (0.750) 93 (0.219)	100 (1.00)	85 (1.00)	100 (0.844) 93 (0.156)	100 (0.906) 92 (0.094)	92 (0.062) 82 (0.844) 53 (0.094)	111 (0.781) 106 (0.188) 93 (0.031)
	8	16	100 (0.719) 93 (0.281)	100 (1.00)	85 (1.00)	100 (0.625) 93 (0.375)	100 (0.812) 92 (0.188)	100 (0.188) 92 (0.250) 82 (0.562)	111 (0.625) 106 (0.375)
CAR	1	15	113 (0.433) 100 (0.567)	112 (0.067) 100 (0.933)	85 (1.00)	100 (0.767) 93 (0.233)	100 (0.367) 92 (0.633)	100 (0.100) 82 (0.900)	111 (0.100) 106 (0.667) 100 (0.200) 98 (0.033)
	2	11	113 (0.545) 100 (0.409) 93 (0.045)	112 (0.045) 100 (0.955)	85 (1.00)	100 (0.500) 93 (0.318) 86 (0.182)	100 (0.636) 92 (0.364)	100 (0.136) 82 (0.864)	111 (0.091) 106 (0.727) 100 (0.045) 98 (0.091) 96 (0.045)
	3	16	113 (0.625) 100 (0.281) 93 (0.094)	100 (1.00)	85 (1.00)	100 (0.656) 93 (0.219) 86 (0.125)	100 (0.625) 92 (0.375)	100 (0.062) 82 (0.938)	111 (0.125) 106 (0.656) 100 (0.156) 98 (0.062)
	4	9	113 (0.667) 100 (0.333)	112 (0.056) 100 (0.944)	85 (1.00)	100 (0.722) 93 (0.278)	100 (0.611) 92 (0.389)	100 (0.111) 82 (0.889)	111 (0.111) 106 (0.667) 100 (0.166) 98 (0.056)
	5	4	113 (0.500) 100 (0.500)	100 (1.00)	85 (1.00)	100 (0.625) 93 (0.375)	100 (0.500) 92 (0.500)	100 (0.500) 82 (0.375) 53 (0.125)	111 (0.375) 106 (0.250) 100 (0.375)
	6	5	113 (0.300) 100 (0.700)	112 (0.100) 100 (0.900)	85 (1.00)	100 (0.800) 92 (0.200)	100 (0.500) 92 (0.500)	100 (0.100) 82 (0.900)	111 (0.800) 106 (0.100) 98 (0.100)
TRN	1	16	113 (0.438) 100 (0.562)	100 (0.313) 91 (0.687)	100 (0.219) 85 (0.781)	104 (0.219) 100 (0.687) 93 (0.094)	100 (0.313) 92 (0.687)	82 (1.00)	111 (0.188) 106 (0.219) 98 (0.562) 96 (0.031)

APPENDIX. Continued.

Site	Patch	*N*	HK	PGM	PGI	TPI	PEP	AAT	LAP
	2	16	113 (0.313) 100 (0.687)	100 (0.469) 91 (0.531)	100 (0.094) 85 (0.906)	104 (0.094) 100 (0.813) 93 (0.094)	100 (0.219) 92 (0.781)	82 (1.00)	111 (0.188) 106 (0.219) 100 (0.062) 98 (0.500) 96 (0.031)
	3	16	113 (0.375) 100 (0.625)	100 (0.313) 91 (0.687)	100 (0.031) 85 (0.969)	104 (0.250) 100 (0.656) 93 (0.094)	100 (0.219) 92 (0.781)	82 (1.00)	111 (0.219) 106 (0.406) 100 (0.094) 98 (0.281)
	4	16	113 (0.281) 107 (0.031) 100 (0.688)	100 (0.281) 91 (0.719)	100 (0.062) 85 (0.938)	104 (0.219) 100 (0.594) 93 (0.188)	100 (0.344) 92 (0.656)	82 (1.00)	111 (0.375) 106 (0.313) 100 (0.031) 98 (0.250) 96 (0.031)
	5	16	113 (0.344) 100 (0.656)	100 (0.344) 91 (0.656)	100 (0.094) 85 (0.906)	104 (0.156) 100 (0.781) 93 (0.063)	100 (0.344) 92 (0.656)	82 (1.00)	111 (0.406) 106 (0.250) 100 (0.031) 98 (0.313)
	6	16	113 (0.344) 100 (0.656)	100 (0.188) 91 (0.813)	100 (0.062) 85 (0.938)	104 (0.281) 100 (0.500) 93 (0.219)	100 (0.469) 92 (0.531)	82 (1.00)	111 (0.313) 106 (0.094) 98 (0.594)
	7	16	113 (0.313) 100 (0.687)	100 (0.125) 91 (0.875)	100 (0.156) 85 (0.844)	104 (0.313) 100 (0.406) 93 (0.281)	100 (0.469) 92 (0.531)	82 (1.00)	111 (0.125) 106 (0.219) 100 (0.031) 98 (0.625)
	8	16	113 (0.250) 100 (0.750)	100 (0.187) 91 (0.813)	100 (0.062) 85 (0.938)	104 (0.375) 100 (0.469) 93 (0.156)	100 (0.438) 92 (0.562)	82 (1.00)	111 (0.156) 106 (0.250) 100 (0.062) 98 (0.500) 96 (0.031)
CSP	1	22	113 (0.114) 107 (0.023) 100 (0.114) 93 (0.750)	100 (0.909) 78 (0.091)	100 (0.159) 85 (0.841)	100 (0.636) 93 (0.204) 86 (0.159)	100 (0.614) 92 (0.386)	100 (0.023) 92 (0.023) 82 (0.955)	111 (0.182) 106 (0.341) 100 (0.045) 98 (0.432)
	2	21	113 (0.024) 100 (0.262) 93 (0.714)	100 (0.881) 78 (0.119)	100 (0.143) 85 (0.833) 68 (0.024)	100 (0.595) 93 (0.238) 86 (0.167)	100 (0.667) 92 (0.333)	82 (1.00)	111 (0.191) 106 (0.309) 100 (0.119) 98 (0.357) 96 (0.024)
	3	18	113 (0.028) 107 (0.028) 100 (0.166) 93 (0.778)	100 (0.778) 78 (0.222)	100 (0.139) 85 (0.833) 68 (0.028)	100 (0.556) 93 (0.361) 86 (0.083)	100 (0.722) 92 (0.278)	100 (0.028) 82 (0.972)	111 (0.167) 106 (0.361) 100 (0.139) 98 (0.333)
	4	7	113 (0.143) 107 (0.071) 100 (0.143) 93 (0.643)	100 (1.00)	100 (0.071) 85 (0.929)	100 (0.786) 93 (0.071) 86 (0.143)	100 (0.786) 92 (0.214)	82 (1.00)	106 (0.500) 100 (0.214) 98 (0.286)
BOD	1	16	100 (0.938) 93 (0.062)	100 (0.656) 91 (0.344)	100 (0.438) 85 (0.562)	100 (0.875) 93 (0.125)	107 (0.219) 100 (0.375) 92 (0.406)	82 (1.00)	111 (0.406) 106 (0.125) 100 (0.156) 96 (0.313)
	2	16	100 (0.875) 93 (0.125)	100 (0.344) 91 (0.656)	100 (0.313) 85 (0.687)	100 (0.562) 93 (0.438)	107 (0.219) 100 (0.500) 92 (0.281)	100 (0.031) 82 (0.969)	111 (0.344) 100 (0.125) 96 (0.531)
	3	16	107 (0.031) 100 (0.781) 93 (0.188)	100 (0.531) 91 (0.469)	100 (0.250) 85 (0.750)	100 (0.844) 93 (0.156)	107 (0.188) 100 (0.500) 92 (0.312)	82 (1.00)	111 (0.375) 106 (0.219) 100 (0.062) 96 (0.344)

APPENDIX. Continued.

Site	Patch	*N*	HK	PGM	PGI	TPI	PEP	AAT	LAP
	4	16	100 (0.844) 93 (0.156)	100 (0.469) 91 (0.531)	100 (0.312) 85 (0.688)	100 (0.781) 93 (0.219)	107 (0.281) 100 (0.438) 92 (0.281)	100 (0.031) 82 (0.969)	111 (0.469) 106 (0.062) 100 (0.250) 96 (0.219)
	5	16	100 (0.812) 93 (0.188)	100 (0.500) 91 (0.500)	100 (0.469) 85 (0.531)	100 (0.781) 93 (0.219)	107 (0.375) 100 (0.281) 92 (0.344)	82 (1.00)	111 (0.500) 106 (0.094) 100 (0.156) 96 (0.250)
	6	16	100 (0.844) 93 (0.156)	100 (0.531) 91 (0.469)	100 (0.281) 85 (0.719)	100 (0.844) 93 (0.156)	107 (0.250) 100 (0.438) 92 (0.312)	82 (1.00)	111 (0.313) 106 (0.094) 100 (0.156) 96 (0.438)
	7	16	107 (0.031) 100 (0.812) 93 (0.156)	100 (0.344) 91 (0.656)	100 (0.250) 85 (0.750)	100 (0.812) 93 (0.188)	107 (0.219) 100 (0.312) 92 (0.469)	82 (1.00)	111 (0.438) 106 (0.031) 100 (0.094) 96 (0.438)
	8	16	100 (0.875) 93 (0.125)	100 (0.219) 91 (0.781)	100 (0.219) 85 (0.781)	100 (0.812) 93 (0.188)	107 (0.125) 100 (0.562) 92 (0.313)	82 (1.00)	111 (0.188) 106 (0.094) 100 (0.125) 96 (0.594)
CRZ	1	16	100 (0.969) 93 (0.031)	100 (0.625) 91 (0.375)	100 (0.781) 85 (0.219)	100 (1.00)	100 (0.625) 92 (0.375)	100 (0.313) 92 (0.469) 82 (0.219)	111 (0.469) 106 (0.281) 100 (0.219) 96 (0.031)
	2	16	100 (0.719) 93 (0.281)	100 (0.781) 91 (0.219)	100 (0.719) 85 (0.281)	100 (1.00)	107 (0.062) 100 (0.469) 92 (0.469)	100 (0.656) 92 (0.250) 82 (0.094)	111 (0.125) 106 (0.250) 100 (0.625)
	3	16	100 (0.938) 93 (0.062)	100 (0.656) 91 (0.344)	100 (0.812) 85 (0.188)	100 (0.938) 93 (0.062)	100 (0.469) 92 (0.531)	100 (0.344) 92 (0.312) 82 (0.344)	111 (0.063) 106 (0.469) 100 (0.469)
	4	16	107 (0.031) 100 (0.875) 93 (0.094)	100 (0.812) 91 (0.188)	100 (0.812) 85 (0.188)	100 (1.00)	100 (0.469) 92 (0.531)	100 (0.313) 92 (0.313) 82 (0.375)	111 (0.281) 106 (0.281) 100 (0.438)
	5	16	100 (0.844) 93 (0.156)	100 (0.687) 91 (0.313)	100 (0.938) 85 (0.062)	100 (1.00)	100 (0.531) 92 (0.469)	100 (0.625) 92 (0.281) 82 (0.094)	111 (0.344) 106 (0.313) 100 (0.344)
	6	16	100 (0.938) 93 (0.062)	100 (0.719) 91 (0.281)	100 (0.719) 85 (0.281)	100 (1.00)	107 (0.031) 100 (0.688) 92 (0.281)	100 (0.250) 92 (0.188) 82 (0.562)	111 (0.313) 106 (0.437) 100 (0.250)
	7	16	100 (1.00)	100 (0.594) 91 (0.406)	100 (0.812) 85 (0.188)	100 (0.938) 93 (0.062)	100 (0.469) 92 (0.531)	100 (0.313) 92 (0.500) 82 (0.188)	111 (0.313) 106 (0.406) 100 (0.281)
	8	16	100 (1.00)	100 (0.500) 91 (0.500)	100 (0.750) 85 (0.250)	100 (1.00)	100 (0.531) 92 (0.469)	100 (0.219) 92 (0.156) 82 (0.625)	111 (0.281) 106 (0.406) 100 (0.313)
MON	1	16	107 (0.031) 100 (0.906) 93 (0.062)	100 (0.062) 91 (0.938)	100 (0.062) 85 (0.938)	100 (0.812) 93 (0.188)	100 (1.00)	82 (1.00)	111 (0.313) 106 (0.469) 100 (0.094) 96 (0.125)
	2	16	100 (0.938) 93 (0.062)	100 (0.094) 91 (0.875) 72 (0.031)	100 (0.219) 85 (0.781)	100 (0.875) 93 (0.125)	100 (1.00)	82 (1.00)	111 (0.188) 106 (0.469) 100 (0.219) 96 (0.125)
	3	16	100 (1.00)	100 (0.531) 91 (0.469)	100 (0.531) 85 (0.469)	100 (0.625) 93 (0.375)	100 (1.00)	82 (1.00)	111 (0.438) 106 (0.562)
	4	16	100 (0.969) 93 (0.031)	100 (0.156) 91 (0.812) 72 (0.031)	100 (0.094) 85 (0.906)	100 (0.938) 93 (0.062)	100 (1.00)	82 (1.00)	111 (0.438) 106 (0.469) 100 (0.062) 96 (0.031)

APPENDIX. Continued.

Site	Patch	*N*	HK	PGM	PGI	TPI	PEP	AAT	LAP
	5	16	100 (0.875) 93 (0.125)	100 (0.094) 91 (0.906)	100 (0.062) 85 (0.938)	100 (0.812) 93 (0.188)	100 (1.00)	100 (0.031) 82 (0.969)	111 (0.313) 106 (0.531) 100 (0.125) 96 (0.031)
	6	16	100 (0.938) 93 (0.062)	100 (0.156) 91 (0.812) 72 (0.031)	100 (0.031) 85 (0.969)	100 (0.813) 93 (0.187)	100 (1.00)	82 (1.00)	111 (0.313) 106 (0.594) 100 (0.062) 96 (0.031)
	7	16	100 (0.969) 93 (0.031)	100 (0.125) 91 (0.875)	100 (0.094) 85 (0.906)	100 (0.875) 93 (0.125)	100 (0.969) 75 (0.031)	82 (1.00)	111 (0.250) 106 (0.594) 100 (0.094) 96 (0.062)
	8	16	100 (1.00)	100 (0.094) 91 (0.906)	100 (0.125) 85 (0.844) 34 (0.031)	100 (0.875) 93 (0.125)	100 (1.00)	82 (1.00)	111 (0.344) 106 (0.375) 100 (0.219) 96 (0.062)
SIM	1	16	100 (0.969) 93 (0.031)	100 (0.031) 91 (0.969)	100 (0.062) 85 (0.906) 74 (0.031)	100 (1.00)	100 (1.00)	82 (1.00)	111 (0.781) 106 (0.125) 100 (0.094)
	2	16	100 (0.781) 93 (0.219)	100 (0.250) 91 (0.750)	100 (0.312) 85 (0.531) 74 (0.156)	100 (1.00)	107 (0.031) 100 (0.969)	100 (0.219) 82 (0.781)	111 (0.812) 106 (0.094) 100 (0.094)
	3	16	100 (0.812) 93 (0.187)	100 (0.406) 91 (0.563) 78 (0.031)	114 (0.062) 100 (0.156) 85 (0.500) 74 (0.281)	100 (1.00)	100 (1.00)	100 (0.250) 82 (0.750)	111 (0.812) 106 (0.188)
	4	16	100 (0.938) 93 (0.062)	100 (0.531) 91 (0.469)	100 (0.250) 85 (0.688) 74 (0.062)	100 (1.00)	100 (1.00)	100 (0.094) 82 (0.906)	111 (0.781) 106 (0.219)
	5	16	100 (0.906) 93 (0.094)	100 (0.094) 91 (0.906)	100 (0.156) 85 (0.813) 74 (0.031)	100 (1.00)	100 (1.00)	100 (0.062) 82 (0.938)	115 (0.031) 111 (0.688) 106 (0.187) 100 (0.094)
	6	16	100 (0.938) 93 (0.062)	100 (0.281) 91 (0.719)	100 (0.031) 85 (0.625) 74 (0.344)	100 (1.00)	107 (0.031) 100 (0.938) 92 (0.031)	100 (0.125) 82 (0.875)	111 (0.594) 106 (0.375) 100 (0.031)
	7	16	100 (0.969) 93 (0.031)	100 (0.281) 91 (0.719)	100 (0.188) 85 (0.781) 74 (0.031)	100 (1.00)	107 (0.031) 100 (0.969)	100 (0.281) 82 (0.719)	115 (0.062) 111 (0.313) 106 (0.250) 100 (0.375)
	8	16	100 (0.781) 93 (0.219)	100 (0.125) 91 (0.875)	114 (0.062) 100 (0.188) 85 (0.687) 74 (0.062)	100 (1.00)	100 (1.00)	100 (0.094) 82 (0.906)	111 (0.750) 106 (0.062) 100 (0.188)
GOL	1	16	100 (0.469) 93 (0.531)	100 (0.094) 91 (0.906)	85 (1.00)	100 (1.00)	100 (1.00)	100 (0.625) 95 (0.031) 82 (0.344)	111 (0.250) 106 (0.563) 100 (0.156) 96 (0.031)
	2	16	100 (0.156) 93 (0.844)	91 (1.00)	85 (1.00)	100 (1.00)	100 (0.906) 97 (0.094)	100 (0.344) 82 (0.656)	111 (0.406) 106 (0.250) 100 (0.250) 96 (0.094)
	3	16	100 (0.281) 93 (0.719)	100 (0.156) 91 (0.844)	85 (1.00)	100 (1.00)	100 (0.938) 97 (0.062)	100 (0.469) 82 (0.531)	115 (0.031) 111 (0.375) 106 (0.344) 100 (0.188) 96 (0.062)
	4	16	100 (0.313) 93 (0.687)	100 (0.031) 91 (0.969)	85 (1.00)	100 (1.00)	100 (0.812) 97 (0.188)	100 (0.625) 95 (0.031) 82 (0.344)	111 (0.281) 106 (0.406) 100 (0.219) 96 (0.094)

APPENDIX. Continued.

Site	Patch	N	HK	PGM	PGI	TPI	PEP	AAT	LAP
	5	16	100 (0.500) 93 (0.500)	100 (0.031) 91 (0.969)	85 (1.00)	100 (1.00)	100 (0.875) 97 (0.125)	100 (0.531) 82 (0.469)	111 (0.375) 106 (0.531) 100 (0.062) 96 (0.031)
	6	16	100 (0.781) 93 (0.219)	100 (0.062) 91 (0.938)	85 (1.00)	100 (1.00)	100 (0.813) 97 (0.187)	100 (0.531) 82 (0.469)	111 (0.313) 106 (0.375) 100 (0.219) 96 (0.094)
	7	16	100 (0.250) 93 (0.750)	91 (1.00)	85 (1.00)	100 (1.00)	100 (0.938) 97 (0.062)	100 (0.812) 82 (0.188)	111 (0.125) 106 (0.500) 100 (0.313) 96 (0.062)
	8	16	100 (0.469) 93 (0.531)	100 (0.125) 91 (0.875)	85 (1.00)	100 (1.00)	100 (0.812) 97 (0.188)	100 (0.625) 82 (0.375)	111 (0.188) 106 (0.531) 100 (0.188) 96 (0.094)
EAN	1	16	100 (0.813) 93 (0.156) 81 (0.031)	100 (0.281) 91 (0.719)	100 (0.156) 85 (0.844)	100 (1.00)	100 (0.938) 92 (0.062)	82 (1.00)	111 (0.281) 106 (0.344) 100 (0.375)
	2	16	100 (0.594) 93 (0.406)	100 (0.344) 91 (0.656)	100 (0.188) 85 (0.812)	100 (1.00)	100 (0.875) 92 (0.125)	100 (0.188) 82 (0.812)	111 (0.062) 106 (0.656) 100 (0.250) 96 (0.031)
	3	16	100 (0.687) 93 (0.313)	100 (0.281) 91 (0.719)	85 (1.00)	100 (1.00)	107 (0.031) 100 (0.875) 92 (0.094)	100 (0.188) 82 (0.812)	111 (0.156) 106 (0.438) 100 (0.219) 96 (0.187)
	4	16	100 (0.781) 93 (0.219)	100 (0.250) 91 (0.750)	100 (0.219) 85 (0.781)	100 (1.00)	100 (0.844) 97 (0.031) 92 (0.125)	100 (0.188) 82 (0.812)	111 (0.094) 106 (0.469) 100 (0.313) 96 (0.125)
	5	16	100 (0.625) 93 (0.375)	100 (0.188) 91 (0.812)	100 (0.156) 85 (0.844)	100 (1.00)	100 (0.906) 92 (0.094)	100 (0.062) 82 (0.938)	111 (0.125) 106 (0.500) 100 (0.219) 96 (0.156)
	6	16	100 (0.719) 93 (0.281)	100 (0.125) 91 (0.875)	100 (0.094) 85 (0.906)	100 (1.00)	100 (0.938) 92 (0.062)	100 (0.375) 82 (0.625)	111 (0.125) 106 (0.594) 100 (0.156) 96 (0.125)
	7	16	100 (0.867) 93 (0.313)	100 (0.313) 91 (0.687)	100 (0.219) 85 (0.781)	100 (1.00)	100 (0.969) 97 (0.031)	100 (0.062) 82 (0.938)	111 (0.094) 106 (0.438) 100 (0.406) 96 (0.062)
	8	16	100 (0.719) 93 (0.281)	100 (0.125) 91 (0.875)	100 (0.094) 85 (0.906)	100 (1.00)	100 (0.969) 92 (0.031)	100 (0.313) 82 (0.687)	111 (0.156) 106 (0.500) 100 (0.250) 96 (0.094)
PTL	1	16	107 (0.062) 100 (0.563) 93 (0.344) 81 (0.031)	100 (0.406) 96 (0.031) 91 (0.563)	100 (0.375) 85 (0.625)	100 (1.00)	102 (0.031) 100 (0.906) 97 (0.062)	100 (0.563) 82 (0.437)	111 (0.438) 106 (0.281) 100 (0.219) 96 (0.062)
	2	16	100 (0.781) 93 (0.219)	100 (0.406) 96 (0.062) 91 (0.531)	100 (0.563) 85 (0.437)	100 (1.00)	102 (0.031) 100 (0.813) 97 (0.062) 92 (0.094)	100 (0.438) 82 (0.562)	111 (0.500) 106 (0.219) 100 (0.188) 96 (0.094)
	3	16	100 (0.687) 93 (0.313)	100 (0.687) 96 (0.031) 91 (0.281)	100 (0.375) 85 (0.625)	100 (1.00)	102 (0.094) 100 (0.781) 92 (0.125)	100 (0.594) 92 (0.062) 82 (0.344)	111 (0.438) 106 (0.500) 100 (0.031) 96 (0.031)

APPENDIX. Continued.

Site	Patch	*N*	HK	PGM	PGI	TPI	PEP	AAT	LAP
	4	16	100 (0.844) 93 (0.156)	100 (0.469) 96 (0.031) 91 (0.500)	100 (0.531) 85 (0.469)	100 (0.969) 93 (0.031)	102 (0.094) 100 (0.812) 92 (0.094)	100 (0.250) 82 (0.750)	111 (0.313) 106 (0.437) 100 (0.156) 96 (0.094)
	5	16	100 (0.687) 93 (0.313)	100 (0.469) 96 (0.031) 91 (0.500)	100 (0.313) 85 (0.687)	100 (1.00)	102 (0.031) 100 (0.906) 92 (0.062)	100 (0.437) 82 (0.563)	111 (0.469) 106 (0.406) 100 (0.094) 96 (0.031)
	6	16	100 (0.687) 93 (0.313)	100 (0.531) 96 (0.031) 91 (0.438)	100 (0.687) 85 (0.313)	100 (0.969) 93 (0.031)	102 (0.031) 100 (0.938) 92 (0.031)	100 (0.344) 82 (0.656)	111 (0.250) 106 (0.500) 100 (0.188) 96 (0.062)
	7	16	107 (0.031) 100 (0.594) 93 (0.375)	100 (0.500) 96 (0.062) 91 (0.438)	100 (0.500) 85 (0.500)	100 (1.00)	102 (0.031) 100 (0.875) 97 (0.062) 92 (0.031)	100 (0.375) 82 (0.625)	111 (0.469) 106 (0.437) 100 (0.094)
	8	16	100 (0.656) 93 (0.344)	100 (0.344) 96 (0.094) 91 (0.562)	100 (0.406) 85 (0.594)	100 (1.00)	102 (0.219) 100 (0.750) 97 (0.031)	100 (0.250) 82 (0.750)	111 (0.500) 106 (0.375) 100 (0.094) 96 (0.031)
PTB	1	16	100 (0.938) 93 (0.062)	116 (0.031) 100 (0.531) 96 (0.094) 91 (0.219) 78 (0.125)	100 (0.125) 85 (0.875)	100 (1.00)	102 (0.156) 100 (0.719) 92 (0.094) 83 (0.031)	100 (0.156) 92 (0.031) 82 (0.813)	111 (0.250) 106 (0.281) 100 (0.313) 96 (0.156)
	2	16	100 (1.00)	116 (0.062) 100 (0.469) 96 (0.125) 91 (0.125) 78 (0.219)	100 (0.219) 85 (0.781)	100 (1.00)	102 (0.187) 100 (0.719) 92 (0.094)	100 (0.125) 82 (0.875)	111 (0.188) 106 (0.531) 100 (0.094) 96 (0.188)
	3	16	100 (0.969) 93 (0.031)	100 (0.625) 91 (0.313) 78 (0.062)	100 (0.219) 85 (0.781)	100 (1.00)	100 (0.844) 92 (0.156)	100 (0.219) 82 (0.781)	111 (0.219) 106 (0.437) 100 (0.188) 96 (0.156)
ISG	1	13	100 (1.00)	116 (0.154) 100 (0.769) 91 (0.077)	100 (0.077) 85 (0.923)	100 (1.00)	100 (0.808) 92 (0.192)	82 (1.00)	106 (0.423) 100 (0.500) 92 (0.077)
	2	6	100 (0.750) 93 (0.250)	100 (0.917) 91 (0.083)	100 (0.083) 85 (0.917)	100 (1.00)	100 (1.00)	82 (1.00)	106 (0.333) 100 (0.583) 92 (0.083)
	3	15	100 (0.833) 93 (0.167)	116 (0.067) 100 (0.767) 91 (0.166)	100 (0.200) 85 (0.800)	100 (1.00)	102 (0.033) 100 (0.967)	100 (0.033) 82 (0.967)	106 (0.633) 100 (0.333) 96 (0.033)
	4	12	100 (0.917) 93 (0.083)	116 (0.042) 100 (0.791) 91 (0.167)	100 (0.208) 85 (0.792)	100 (1.00)	102 (0.042) 100 (0.958)	100 (0.042) 82 (0.958)	111 (0.042) 106 (0.583) 100 (0.333) 96 (0.042)