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HABITAT CONTINUITY AND THE GENETIC STRUCTURE OF *DROSOPHILA* POPULATIONS

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The evolutionary cohesiveness of a species depends to a large extent on the degree to which its populations are connected via gene flow. As a consequence, patterns and levels of gene flow can affect both microevolutionary processes, including mechanisms of adaptation and speciation (Fisher

1930; Wright 1977; Barton 1989), and macroevolutionary patterns of stasis and change (Futuyma 1987). For example, Fisher's (1930) and Wright's (1977) alternative models of adaptive evolution differ in two basic respects: the importance of epistatic interactions among genes and the levels of

gene flow among conspecific populations. While Fisher viewed species as essentially panmictic, Wright's shifting balance theory requires limited gene flow among small, semi-isolated populations so that different populations arrive at different peaks in the adaptive landscape.

Animal species vary considerably in such factors as philopatry, individual vagility, geographical distance between populations, and habitat continuity (Baker 1978), and these factors may have substantial effects on levels of gene flow among populations and thus the means by which these species undergo adaptive evolution (Avisé 1994). Several recent studies have examined the effects of habitat or resource subdivision on population genetic structure, with the *a priori* prediction that species restricted to patchily distributed habitats experience greater restriction in gene flow among geographically separated populations than those utilizing continuously distributed habitats (McCauley and Eanes 1987; Rank 1992). However, because these studies considered individual species, it is difficult to distinguish the effects of habitat structure per se from other possible effects on genetic differentiation. In the present paper, we examine the population genetic structure of three closely related species of *Drosophila* flies to test the generality of the predicted relationship between habitat continuity and population genetic structure.

The *Drosophila* species we chose for this study—*D. falleni*, *D. recens*, and *D. quinaria*—include all of the widespread members of the *D. quinaria* species group in the northeastern United States and eastern Canada. These species differ considerably in the spatial distributions of their preferred breeding sites (Spencer 1942; Wheeler 1960): *D. falleni* and *D. recens* utilize mushrooms as their principal breeding sites, while *D. quinaria* is restricted to the decaying fruits and leaves of skunk cabbages, *Symplocarpus foetidus* (Jaenike 1978; Lacy 1984).

Because almost all of the Northeast originally was forested (Kuchler 1975) and much of it still is, and because mushrooms occur in association with most trees of temperate forests (Orlos 1975), suitable habitat for *D. falleni* and *D. recens* is essentially continuous in distribution. In contrast, skunk cabbages grow as extensive, but discrete patches in wet, low-lying areas such as swamps and floodplains (Wilén and Tiner 1993; Redington 1994). A related species, the yellow skunk cabbage (*Lysichitum americanum*) is so restricted to such areas that it is an excellent indicator of average water-table depth (Minore 1969). Patches of *S. foetidus* are typically separated by large intervening areas of drier habitat lacking skunk cabbage, and these may serve as barriers to dispersal for *D. quinaria*. Skunk cabbage patches are persistent features of the environment; the ones from which we collected *D. quinaria* in New York and Maine have persisted in the same sites for over 20 years (JJ pers. obs.).

If habitat continuity has a discernible effect on the genetic structure of populations, then *D. quinaria*, a species restricted to a patchily distributed habitat, should exhibit greater levels of population differentiation than either *D. falleni* or *D. recens*, which utilize ubiquitous mushroom hosts. We employed markers of both the nuclear (allozymes) and mitochondrial genomes to assess the population structure of these three species to test this predicted relationship between habitat

TABLE 1. Collecting localities and sample sizes for *Drosophila falleni*, *D. recens*, and *D. quinaria*. Regions include all sites within a given county. Collection periods indicated in superscripts: ¹June 1995; ²August 1995; ³June through August 1995.

Site	Species (n)		
	<i>D. falleni</i>	<i>D. recens</i>	<i>D. quinaria</i>
Mendon Ponds Park, Monroe Co., New York	60 ¹	46 ³	
Long Meadow Circle, Monroe Co., New York			28 ³
Turk Hill Road, Monroe Co., New York			21 ³
Big Moose Lake, Herkimer Co., New York	30 ¹	31 ³	
Brunswick Pines, Cumberland Co., Maine	42 ²	28 ²	
Dunham's Point, Deer Isle, Hancock Co., Maine	30 ²		28 ²
Water Pump, Deer Isle, Hancock Co., Maine			30 ²
Pymatuning Laboratory of Ecology, Crawford Co., Pennsylvania	29 ²		30 ²

continuity and genetic population structure. We employed markers from both the nuclear and mitochondrial genomes because they are differentially susceptible to variation in effective population size and gene flow. Mitochondrial DNA haplotypes are more sensitive to the effects of genetic drift because the effective population size for mtDNA is approximately one-quarter that of nuclear genes (Avisé 1994). In addition, mtDNA markers are expected to be less sensitive than nuclear genes to the homogenizing effects of gene flow among populations, because dispersing males make no contribution to mitochondrial gene flow, and dispersing inseminated females carry only one mtDNA haplotype but four or more sets of nuclear genes (Hale and Singh 1991; Avisé 1994).

MATERIALS AND METHODS

Collection of Flies

Drosophila falleni and *D. recens* were collected by sweep-netting over mushroom (*Agaricus bisporus*) baits placed within forests, and *D. quinaria* were collected by sweeping over cucumber baits set out within patches of the skunk cabbage *Symplocarpus foetidus*. Collections were made during the summer of 1995 at the localities indicated in Table 1. Samples were returned to the laboratory and frozen at -80°C pending allozyme and mtDNA analyses. Because we collected adult flies, rather than flies bred from either mushrooms or skunk cabbages, we regard each fly as an independent sample of the genetic variation present within a local population. Because females tend to lay clutches of eggs on breeding sites, individuals bred from a single breeding site may not represent genetically independent samples (Jaenike and Selander 1979; Jaenike and James 1991).

Allozyme Methods

We used starch gel electrophoresis to determine the genotypes of every fly at six to seven polymorphic loci. Genotypes at the following polymorphic loci were scored in

each species: *D. falleni*: *Acp*, *Est*, *Lap*, *Mdh-1*, *Mdh-2*, *Pep-1*, and *Pgm*; *D. recens*: *Est*, *Lap*, *Mdh-1*, *Mdh-2*, *Pep-2*, and *Pgm*; *D. quinaria*: *Acp*, *Est*, *Lap*, *Mdh-1*, *Mdh-2*, *Pep-2*, and *Pgm*. Details of electrophoretic methods are described elsewhere (Jaenike and Selander 1979; Jaenike 1989).

Mitochondrial DNA Methods

Total genomic DNA was isolated from the same individual flies used for the allozymes. DNA isolation procedures followed those of Shoemaker and Ross (1996). A 4-kb portion of the mitochondrial DNA (mtDNA) genome (including the A+T-rich noncoding region) was PCR amplified using the conserved primers C1-N-1560 (Nancy) and SR-J-14612 (12sair; see Simon et al. [1994] for sequence of primers). PCR reactions were performed in 100 μ L volumes containing 10 \times buffer; 0.1 mM each of dATP, dCTP, dTTP, and dGTP; 3.5 mM magnesium chloride; 25 pmol of each primer; 100 ng of template DNA, and 5 U of *Taq* DNA polymerase. Amplifications were carried out in a thermal cycler programmed as follows: 1 min at 94°C for one cycle; 30 sec at 94°C, 1 min at 45°C, and 4.5 min at 68°C for 30 cycles; 5 min at 72°C for one terminal cycle. Aliquots of the 4-kb PCR products then were digested with the restriction enzymes *Apa* I, *Ase* I, *Bam*H I, *Bsr* I, *Bst*U I, *Eco*R I, *Hha* I, *Hinc* II, *Hind* III, *Hinf* I, *Pst* I, *Rsa* I, *Ssp* I, and *Taq* I (5–8 μ L per reaction). Digestion products were electrophoresed in 1.75% agarose gels, stained with ethidium bromide, and visualized under UV light; stained gels were photographed using Polaroid 667 film. The presence or absence of restriction sites inferred using complete and partial digestion procedures defined the composite haplotypes.

Data Analyses

The observed genotype frequencies for each allozyme locus within each population were compared to those expected under Hardy-Weinberg equilibrium (HWE) using the programs GENEPOP (Raymond and Rousset 1995). This program tests for departures from HWE using Fisher's exact test (Louis and Dempster 1987; Weir 1990). We used a sequential Bonferroni procedure to evaluate the statistical significance of each test because of the large number conducted (Hochberg 1988). Expected heterozygosities at *Est*, *Lap*, *Mdh-1*, *Mdh-2*, and *Pgm* were calculated following equation 8.4 of Nei (1987).

Genetic differentiation among populations of each species was assessed in two ways. First, the probabilities associated with the observed allelic differentiation among populations were computed for each locus using a Markov chain procedure to estimate Fisher's exact probability, with the joint probabilities over all loci obtained using Fisher's combined probability test (Raymond and Rousset 1995). These procedures were implemented using the computer program GENEPOP (Raymond and Rousset 1995). Second, values of the fixation index F_{ST} were estimated using the methods of Weir and Cockerham (1984). Variances for each locus were obtained by jackknifing over alleles (when possible), and summary estimates of F -statistics and their variances were obtained by jackknifing over loci (95% CIs were generated by assuming the t -distribution). All rare alleles (< 5% frequency

in every population) and the second most common allele at each locus were excluded from these analyses because of the nonindependence of frequency data.

Mitochondrial DNA haplotype frequencies were estimated in each population of the three species using all sampled individuals. For all three species, haplotype differentiation was first analyzed among populations in different regions (counties) listed in Table 1. For the one species showing significant among-population differentiation at this level, *D. quinaria*, we then analyzed variation at two spatial scales: among populations in different skunk cabbage patches within regions and among pooled populations in different regions. These analyses were done using the AMOVA approach (i.e., analysis of molecular variance) of Excoffier et al. (1992), as implemented in the program WINAMOVA. This method partitions haplotype diversity within and between differing levels of hierarchical population subdivision, yielding analogs to F -statistics designated as Φ -statistics (Michalakis and Excoffier 1996).

Two measures of distance between restriction-site haplotypes were employed in these analyses, the equidistant metric, which assumes that all haplotypes observed are equally divergent from one another, and the Euclidean metric, which equals the number of restriction site differences between haplotypes (e.g., Nei and Tajima 1981). The probabilities that the values of Φ_{ST} at the various hierarchical levels are significantly positive (indicating differentiation among populations) were determined by permutation analysis using 1,000 randomly permuted distance matrices (Excoffier et al. 1992).

RESULTS

Allozyme and mtDNA Variation

A two-way analysis of variance (PROC GLM; SAS Institute 1994) of the five nuclear loci assayed in all three *Drosophila* species—*Est*, *Lap*, *Mdh-1*, *Mdh-2*, and *Pgm*—reveals that the mean within-population expected heterozygosity varied significantly among loci, when pooled across species, ranging from 0.02 in *Mdh-2* to 0.64 in *Est* ($P < 0.0001$; Table 2). Mean within-population heterozygosity varied significantly among all three *Drosophila* species, with *D. quinaria* exhibiting the lowest level of variation ($H_{exp} = 0.21$), *D. falleni* the greatest ($H_{exp} = 0.41$), and *D. recens* intermediate ($H_{exp} = 0.35$).

Observed genotype frequencies were significantly different from HWE in only 3% (2 of 61) of the comparisons. In both cases—*Pgm* in *D. falleni* at Pymatuning and *Pep-1* in *D. quinaria* at Long Meadow Circle—there were fewer heterozygotes than expected.

The total number of mitochondrial haplotypes varied dramatically among the three species, ranging from 10 in *D. recens* to 42 in *D. falleni*. *Drosophila quinaria* was intermediate, with 23 distinct haplotypes.

Population Genetic Structure: Allozymes

Exact tests of allele frequency differentiation among populations were not significant at any locus for *D. recens* and at only a single locus for *D. falleni* (*Est*). In contrast, allele frequency differentiation was significant in *D. quinaria* at

TABLE 2. Average H_{exp} in populations of *Drosophila falleni*, *D. recens*, and *D. quinaria* based on five common allozyme loci.

Species/population	H_{exp}					Mean
	<i>Est</i>	<i>Lap</i>	<i>Mdh-1</i>	<i>Mdh-2</i>	<i>Pgm</i>	
<i>D. falleni</i>						
Mendon Ponds Park	0.83	0.64	0.05	0.00	0.51	0.41
Big Moose Lake	0.80	0.62	0.00	0.00	0.55	0.39
Pymatuning	0.84	0.62	0.09	0.00	0.55	0.42
Brunswick Pines	0.82	0.56	0.07	0.00	0.66	0.42
Dunham's Point	0.80	0.63	0.00	0.03	0.55	0.40
<i>D. recens</i>						
Mendon Ponds Park	0.59	0.60	0.19	0.00	0.38	0.35
Big Moose Lake	0.59	0.55	0.38	0.09	0.32	0.39
Brunswick Pines	0.55	0.39	0.12	0.06	0.36	0.30
<i>D. quinaria</i>						
Long Meadow/Turk Hill	0.35	0.25	0.17	0.00	0.19	0.19
Water Pump/Dunham's Point	0.46	0.24	0.00	0.00	0.18	0.28
Pymatuning	0.46	0.39	0.27	0.03	0.22	0.18
Mean	0.65	0.50	0.12	0.02	0.41	

four out of seven loci (*Acph*, *Est*, *Lap*, and *Mdh-1*). The overall level of genetic differentiation within *D. quinaria* was highly significant when all seven loci were considered together ($\chi^2 = 43.8$, $df = 12$, $P < 10^{-5}$). Neither summary value for *D. recens* or *D. falleni* was significant ($\chi^2 = 17.1$, $df = 12$, $P = 0.15$ and $\chi^2 = 21.1$, $df = 14$, $P = 0.10$, respectively).

TABLE 3. Estimates of Wright's F -statistics (mean \pm SE) for *Drosophila falleni*, *D. recens*, and *D. quinaria* populations in different regions. Single-locus values were obtained by jackknifing over alleles (when possible) and summary values were obtained by jackknifing over loci.

Locus	F_{IT}	F_{ST}	F_{IS}
<i>Drosophila falleni</i>			
<i>Acph</i>	-0.0169	0.0110	-0.0282
<i>Est</i>	0.0420 \pm 0.0400	0.0048 \pm 0.0057#	0.0376 \pm 0.0432
<i>Lap</i>	0.2109 \pm 0.0019*	-0.0110 \pm 0.0160	0.2195 \pm 0.0006*
<i>Mdh-1</i>	0.2216	0.0002	0.2214
<i>Mdh-2</i>	0.0207	0.0025	0.0183
<i>Pep-1</i>	-0.0193	0.0212	-0.0414
<i>Pgm</i>	0.2547	0.0235	0.2368
Summary	0.1110 \pm 0.0658	0.0040 \pm 0.0067	0.1076 \pm 0.0672
<i>Drosophila recens</i>			
<i>Est</i>	-0.0852 \pm 0.0295*	-0.0044 \pm 0.0176	-0.0795 \pm 0.0492
<i>Lap</i>	0.1437 \pm 0.0875	0.0434 \pm 0.0285	0.1092 \pm 0.1128
<i>Mdh-1</i>	0.1235	0.0343	0.0923
<i>Mdh-2</i>	-0.0151	0.0193	-0.0351
<i>Pep-2</i>	0.1946 \pm 0.1623	-0.0115 \pm 0.0064	0.2051 \pm 0.1639
<i>Pgm</i>	-0.0113 \pm 0.1507	-0.0105 \pm 0.0230	-0.0002 \pm 0.1508
Summary	0.0853 \pm 0.0814	0.0049 \pm 0.0111	0.0807 \pm 0.0798
<i>Drosophila quinaria</i>			
<i>Acph</i>	-0.0483 \pm 0.0780	0.0231 \pm 0.0313#	-0.0748 \pm 0.0471
<i>Est</i>	-0.0273 \pm 0.0129*	0.0082 \pm 0.0128#	-0.0352 \pm 0.0263
<i>Lap</i>	0.1330	0.0066#	0.1272
<i>Mdh-1</i>	-0.0302	0.0801#	-0.1200
<i>Mdh-2</i>	0.0248	0.0102	0.0148
<i>Pep-2</i>	0.2467 \pm 0.0694*	-0.0023 \pm 0.0019	0.2485 \pm 0.0702*
<i>Pgm</i>	0.1312 \pm 0.0812	-0.0105 \pm 0.0100	0.1401 \pm 0.0790
Summary	0.0440 \pm 0.0625	0.0168 \pm 0.0083*	0.0282 \pm 0.0705

* Values differ significantly from zero.

Indicates significant genetic structure among populations using an exact test of differentiation.

Our estimates of genetic differentiation for each species using Wright's F -statistics yielded similar results. Summary F_{ST} -values were not significantly different from zero for either *D. recens* ($F_{ST} = 0.0049$) or *D. falleni* ($F_{ST} = 0.0040$), whereas this value for *D. quinaria* ($F_{ST} = 0.0168$), although small, was significantly greater than zero (Table 3). Thus, for nuclear markers neither of the two mycophagous *Drosophila* species exhibited significant genetic differentiation among populations, while populations of *D. quinaria* did show slight but significant geographical differentiation. However, summary F_{ST} -values for each of the three species were not significantly different from each other.

Population Genetic Structure: mtDNA

Analyses of population differentiation using mtDNA haplotype frequencies revealed patterns qualitatively similar to those found for nuclear markers (Table 4). No significant differentiation among populations was observed in either *D. falleni* or *D. recens*, with over 99% of the haplotype diversity residing within populations. In contrast, populations of *D. quinaria* differed significantly in haplotype frequencies ($\Phi_{ST} = 0.018$; $P < 0.001$; Table 4). Although statistically significant, the magnitude of population differentiation in this species was quite low, explaining only 2.2% of the total mitochondrial diversity.

A hierarchical analysis of molecular variation in *D. quinaria* revealed that the differentiation observed among populations was attributable solely to differentiation among populations in different skunk cabbage patches within geograph-

TABLE 4. Analysis of molecular variance (AMOVA) for mtDNA haplotypes in *Drosophila falleni*, *D. recens*, and *D. quinaria* in the northeastern United States. The equidistant metric assumes that all haplotypes are equally divergent from one another, whereas the Euclidean metric is a distance measure that equals the number of restriction site differences between haplotypes. The total haplotypic variance is partitioned among three levels for *D. quinaria* in the bottom part of the table: between regions, among habitat patches within regions, and within habitat patches. The statistic Φ_{CT} measures the extent of differentiation between regions, Φ_{SC} measures differentiation among habitat patches within each region, and Φ_{ST} measures differentiation among all habitat patches. The probabilities (P) that the estimates of the Φ -statistics do not differ from zero (no differentiation) were determined by permutation analysis using 1000 randomly permuted data matrices.

Variance component	Equidistant metric			Euclidean metric		
	% of total variance	Φ -statistics	P	% of total variance	Φ -statistics	P
<i>Drosophila falleni</i>						
Among regions	0.9	$\Phi_{ST} = 0.008$	0.134	0.0	$\Phi_{ST} = 0.000$	0.623
Within regions	99.1			100.0		
<i>Drosophila recens</i>						
Among regions	0.0	$\Phi_{ST} = 0.000$	0.672	0.0	$\Phi_{ST} = 0.000$	0.769
Within regions	100.0			100.0		
<i>Drosophila quinaria</i>						
Among regions	2.2	$\Phi_{ST} = 0.022$	0.051	1.8	$\Phi_{ST} = 0.018$	< 0.001
Within regions	97.8			98.2		
<i>Drosophila quinaria</i>						
Among regions	0.0	$\Phi_{CT} = 0.000$	0.579	0.7	$\Phi_{CT} = 0.007$	0.298
Among habitat patches within regions	6.4	$\Phi_{SC} = 0.063$	0.011	1.7	$\Phi_{SC} = 0.017$	0.019
all habitat patches	6.4	$\Phi_{ST} = 0.045$	0.004	2.4	$\Phi_{ST} = 0.024$	< 0.001
Within habitat patches	93.6			97.6		

ical regions (Table 4). Indeed, genetic differentiation among the populations in patches separated by less than 20 km accounts for 6.4% of the total haplotype diversity, with all remaining variation accounted for by variation within patches. Such differentiation between populations inhabiting different local skunk cabbage patches was not observed for nuclear markers, using either GENEPOP or hierarchical F -statistics.

DISCUSSION

The patterns of genetic differentiation among populations of the three *Drosophila* species were consistent with the prediction that levels of gene flow among *Drosophila* populations depend on the spatial structure of a species' habitat. Essentially no detectable genetic differentiation was found among populations of either *D. falleni* or *D. recens*, which, being mycophagous, occupy continuously distributed forest habitat. This lack of genetic differentiation was observed for both nuclear and mitochondrial markers in these two species. The lack of differentiation of mitochondrial markers, which are less sensitive to the homogenizing effects of interpopulation dispersal, suggests that gene flow among these populations is extensive.

In contrast, *D. quinaria*, which utilizes patchily distributed skunk cabbages as breeding sites, did exhibit significant differentiation among populations for both classes of genetic markers, supporting our prediction that a species occupying a patchily distributed habitat should exhibit greater differentiation among populations than a species that is continuously distributed. We must qualify this conclusion in several respects. First, although the estimated summary F_{ST} -value was three to four times greater in *D. quinaria* than in either

D. falleni or *D. recens*, this difference is not significant, as indicated by the wide confidence limits around these values (Table 3). For such low values of F_{ST} —the greatest was 0.017 in *D. quinaria*—our tests have little power to reveal statistical differences. Second, we have sampled only a single patchily distributed species (*D. quinaria*), whereas a statistically robust approach would require the inclusion of several evolutionarily independent comparisons (Harvey and Pagel 1991). This is not possible in our case, however, because the *D. quinaria* species group, to which we have restricted our analysis, does not contain any other geographically widespread species in the northeastern United States. Finally, because mtDNA haplotypes essentially represent alleles at a single locus, variation among species in levels of mtDNA differentiation are more subject to locus-specific stochastic events than are differences in overall F_{ST} for a set of nuclear loci. Nevertheless, the finding that *D. quinaria* exhibited greater geographic differentiation at both nuclear and mitochondrial loci suggests that something other than locus-specific stochastic effects is the cause of this differentiation.

Although significant genetic differentiation was observed among populations of *D. quinaria*, the level of differentiation, as revealed by both F_{ST} and Φ_{ST} estimates, was surprisingly low. Indeed, estimates of Nm derived from our F_{ST} -values for *D. quinaria* are on the order of 10 to 30, indicating considerable gene flow among populations despite their isolation from each other by considerable stretches of unsuitable breeding habitat.

Our hierarchical estimates of mtDNA population structure in *D. quinaria* indicate that the slight differences observed among populations are due primarily to differentiation among habitat patches within geographical regions. Such local, but

not regional, differentiation might result if the dispersal distance of *D. quinaria* is highly leptokurtic, so that the migrants into a given skunk cabbage patch are as likely to come from distant as from nearby populations. Alternatively, small population sizes within patches could lead to relatively rapid divergence due to drift, even among populations within a single geographical region. Having one-quarter the effective population size of nuclear genes, mtDNA haplotypes will be especially susceptible to drift in small populations.

Our data, therefore, suggest that all three of these species are nearly panmictic and probably undergo adaptive evolution in a manner more along the lines proposed by Fisher (1930) than Wright (1977), even though *D. quinaria* has an ecological population structure that would appear conducive to a shifting balance process. This conclusion must be tempered by the realization that the shifting balance model does not require that all populations in a species experience low levels of gene flow; it is sufficient that only a subset of them exhibit substantial isolation (Coyne et al. 1997). If any populations of *D. falleni*, *D. recens*, or *D. quinaria* are highly isolated, we did not encounter them in our study.

The inferred high rates of gene flow among populations of *D. falleni* and *D. recens* may appear to be inconsistent with the relatively low observed rates of local dispersal in mycophagous *Drosophila* (Montague 1985; Worthen 1989). For example, Montague (1985) found mean dispersal rates on the order of 10 m to 15 m per day in an experimental field study of *D. falleni*. One might expect that such low vagility would limit gene flow among distant geographic populations, regardless of habitat structure. However, these observed rates of local dispersal probably underestimate interpopulation gene flow, because wind-borne, long-distance migrants were not sought in these studies. Therefore, the apparent discrepancy between direct measures of dispersal rates and indirect estimates of gene flow in these species may reflect the sensitivity of populations to the homogenizing effects of rare long-distance migrants (Slatkin 1989; see also Kot et al. 1996).

Previous studies of the genetic population structure of species occupying patchily distributed habitats have revealed higher levels of differentiation than we found in *D. quinaria*. For instance, populations of the butterfly *Euphydryas editha*, the milkweed beetle *Tetraopes tetraophthalmus*, and the leaf beetle *Chrysomela aeneicollis*, all of which occupy patchy habitats, exhibit F_{ST} -values of 0.12, 0.15, and 0.13, respectively, compared to only 0.017 in *D. quinaria* (Pashley et al. 1985; McCauley and Eanes 1987; Rank 1992). Indeed, the level of population differentiation in *D. quinaria* is more similar to that of its close relatives *D. falleni* and *D. recens*, whose habitats are continuously distributed, than to other unrelated species occupying patchy habitats.

This difference between *D. quinaria* and these other patchily distributed species in levels of population differentiation may result from variation in the vagility of the these organisms across regions of unsuitable habitat. For example, the intervening areas between patches of preferred habitat may vary in their suitability to different species. Although *D. quinaria* never breed in mushrooms (Lacy 1984; Jaenike, unpubl. data), they may be able to utilize certain forest resources that, although inadequate to sustain a population of

D. quinaria indefinitely, could provide a bridge between populations occupying different skunk cabbage patches. Resources for which the basic reproductive rate, R_0 , is less than one, but greater than zero might serve this purpose. For instance, one of us (JJ) has found *D. quinaria* breeding in slime fluxes within forests on rare occasions.

Alternatively, levels of gene flow among populations of patchily distributed species may be phylogenetically conservative. Perhaps *Drosophila* flies are more likely than beetles or butterflies to be borne aloft by winds and dispersed long distances. Interestingly, a comparison of F_{ST} -values reported for several species of Diptera and Coleoptera (McCauley and Eanes 1987) indicates that flies, in general, tend to be characterized by lower levels of F_{ST} than beetles. If genetic population structure is evolutionarily conservative, then the processes of adaptive evolution may depend as much on the phylogenetic position of a species as on the details of its present population structure, that is, be a clade-level property (Williams 1992).

In summary, our genetic data show that populations of the three *Drosophila* species we investigated, the continuously distributed *D. falleni* and *D. recens* and the patchily distributed *D. quinaria*, exhibit low levels of genetic differentiation. These findings suggest that all three of these species experience high levels of gene flow among populations and, therefore, probably do not evolve by a shifting balance process. However, for loci that exhibit strong epistatic interactions, such as those involved in sex-ratio meiotic drive and its suppression (Jaenike 1996), current levels of gene flow in *D. quinaria* may be sufficiently low to allow populations in different habitats to evolve toward different adaptive peaks.

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