DISPERSAL OF INVERTEBRATES AMONG TEMPORARY PONDS: ARE GENETIC ESTIMATES ACCURATE?

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ABSTRACT

Dispersal is difficult to quantify in most temporary pond invertebrates. As a consequence, researchers often infer the movement of individuals from indirect estimates of gene flow. Here, we review the assumptions associated with the most common gene flow estimate, which approximates migration using a simple island model and assumes equilibrium. Particular attention is focused on empirical studies of temporary pond invertebrates, where nonequilibrium conditions may be particularly important. When populations have not reached a long-term equilibrium between the migration of genes and random drift, estimates of movement can be biased dramatically. Eight comparative and theoretical tests for ascertaining these biases are described here. In some cases, specific hypotheses regarding contemporary and historical processes can also be tested statistically using computer simulations. Although the accuracy of gene flow estimates in temporary pond species varies widely, empirical and theoretical methods for assessing this accuracy are almost always available.

INTRODUCTION

Freshwater invertebrates provide ideal systems for quantitative studies of dispersal (De Meester, 1996). Lakes, ponds, and rivers possess relatively discrete boundaries, suggesting that problems defining populations should be minor. The relative ease with which zooplankton and benthic invertebrates can be sampled has led to a wealth of distributional information. Individuals are usually numerous enough that population-level parameters can be estimated without severely impacting the system. Finally, studies of pond-dwelling invertebrates can be generalized to organisms in other ephemeral or fluctuating habitats (e.g., Mort, 1991; Wissinger, 1997).

However, the dispersal of freshwater organisms is not well understood. Zooplankton adults are particularly tiny, short-lived, and difficult to study behaviorally in nature. Dormant eggs, which are thought to be the primary dispersive stage in many taxa, are even smaller and more difficult to sample and identify. Direct studies of colonization are rare (Maguire, 1963; Barnes, 1983; Jenkins and Buikema, 1998). As a result, freshwater

biologists tend to invoke dispersal vectors such as vertebrates, wind, and tornadoes, based primarily on anecdotal observations, circumstantial evidence, and indirect experimentation (e.g., Bajkov, 1949; Talling, 1951; Mitchell, 1962; Moore and Faust, 1972; Peck, 1975). The role of ducks and other waterfowl has become firmly entrenched (Darwin, 1859; Proctor, 1964; Boag, 1986; Saunders et al., 1993), so that it is often difficult to imagine other ways for freshwater invertebrates to disperse. With the exception of disease-carrying species of mosquitoes, there are also surprisingly few quantitative studies of dispersal in freshwater insect adults.

For these reasons, estimates of gene flow would seem to be a panacea for researchers interested in the movement of freshwater invertebrates among temporary ponds. Genetic similarities among populations are expected to reflect dispersal qualitatively and, under certain assumptions, quantitatively (Slatkin, 1985). Gene flow estimates also have a number of advantages over ecological estimates of dispersal. For example, they constitute long-term averages and account for successful breeding of dispersers into the new gene pool. The ease with which gene flow can be estimated in novel species is reflected by the steady accumulation of studies on a diversity of taxa. New reviews on the topic are available annually across a variety of subdisciplines (Slatkin, 1994; Palumbi, 1995; Roderick, 1996; Neigel, 1997; Bossart and Prowell, 1998; Bohonak, 1999a).

However, the potential benefits of using these techniques are balanced by a large number of caveats. There is no general agreement on (1) the best way to estimate population subdivision (usually as F_{st} or a related statistic) and gene flow (usually as $N_e m$, the number of migrants per generation), (2) the accuracy of these estimates, or even (3) their general utility (Larson et al., 1984; Weir and Cockerham, 1984; Nei, 1986; De Meester, 1996; Bohonak et al., 1998; Bossart and Prowell, 1998; Peterson and Denno, 1998b; Bohonak, 1999a; Whitlock and McCauley, 1999). Here, we examine these issues with particular focus on temporary pond invertebrates. By extension, a majority of the discussion is applicable to organisms inhabiting other ephemeral habitats. First, we explicitly list the major underlying assumptions of the most widely used estimate of gene flow. The rationale for assuming that populations have reached an equilibrium state is discussed in detail, as are ways in which this assumption might be tested. Finally, we address the questions most relevant to population biologists: Is the assumption of equilibrium realistic? When are gene flow estimates accurate?

GENE FLOW AS ESTIMATED FROM THE ISLAND MODEL

Genetic population differentiation (F_{st}) is usually translated into gene flow using the island model of Sewall Wright (Wright, 1931, 1969; general treatments of this and other methods for estimating gene flow can be found in Slatkin, 1985; Roderick, 1996; Weir, 1996; Neigel, 1997). In population genetics terminology, N_e denotes the genetic effective size of each population and m is the migration rate (i.e., the per capita rate at which genes move from one population to another; see Hartl and Clark, 1989). The product of these ($N_e m$) is the number of migrants per generation leaving and entering each population. (Note that the migration rate m cannot be estimated from F_{st} directly. As a result,

the terms *gene flow* and *migration* often refer to the quantity $N_e m$ in empirical population genetic studies.) Because F_{ST} ranges from 0 (all populations identical) to 1 (populations are maximally differentiated), it is inversely proportional to the amount of gene flow.

Eventually, an equilibrium may be reached in which the loss of variation within populations from random drift is balanced by the introduction of novel alleles through migration. When F_{ST} has reached this equilibrium, its value ceases to change directionally over time. Using the island model and assuming that m is relatively small, gene flow can be estimated from the equilibrium expectation:

$$N_e m = \frac{1}{4} \ (F_{ST}^{-1} - 1) \tag{1}$$

It is this value that most researchers use as a surrogate for dispersal (in an ecological sense). Although not always made explicit, the estimation of gene flow in this way incorporates the following six assumptions (see Slatkin, 1985; Daly, 1989; Hartl and Clark, 1989; Whitlock and McCauley, 1999).

ASSUMPTION 1: THE SAMPLED POPULATIONS ARE A RANDOM SUBSET OF AN INFINITE SET OF POPULATIONS

The equations underlying the island model assume that immigrating alleles are different from resident alleles. This will occur when populations are, for the most part, genetically isolated from one another. Although this may seem reasonable for a very large assemblage of populations with little gene flow, species with very limited distributions may violate this assumption. Theoretical expectations and computer simulations for the "finite island model" suggest that the bias from using eq 1 is not pronounced unless there are very few interacting populations ($\approx$ 5 or less; Takahata and Nei, 1984; Chakraborty and Leimar, 1987; Cockerham and Weir, 1987). As with any statistic, extrapolating conclusions beyond the sampled populations implicitly assumes that the sampled populations are a randomly chosen subset.

ASSUMPTION 2: POPULATIONS ARE PERMANENT, OF EQUAL SIZE, AND HAVE EQUAL MIGRATION RATES

Obviously, assumptions of permanence, equal size, and equal migration rates are violated in almost every natural system. Unfortunately, the consequences for gene flow estimates are diverse and not always predictable. For example, extinctions and recolonizations can either promote or suppress the divergence of populations, depending on the nature of the migrant pool and the number of colonists that found new populations (Slatkin, 1977; Wade and McCauley, 1988; Whitlock and McCauley, 1990; Whitlock, 1992; Follett and Roderick, 1996). Even in the absence of local extinctions, differences among populations in N_e or m can bias gene flow estimates because of the nonlinear relationship between F_{ST} and $N_e m$ (discussed in Whitlock, 1992; Whitlock and McCauley, 1999). Temporal demographic fluctuations can also lead to fluctuations in estimates of $N_e m$ (Whitlock, 1992), perhaps invalidating its use as a stable, long-term average of gene flow.

To determine the importance of these processes in any system requires an under-
standing of the demography of colonization events and the frequency of population
extinctions. In general, it will also be important to know the degree to which coloniza-
tion and dispersal involve similar or distinct processes (see discussions in Slatkin, 1985;
Wade and McCauley, 1988). For example, De Meester (1996) discusses how local
adaptation in *Daphnia* may decouple gene flow between established populations and
colonization of new habitats.

ASSUMPTION 3: MUTATION RATES ARE NEGLIGIBLE

Equation 1 assumes that the per generation mutation rate μ is very low relative to the
migration rate *m*. Because the resulting bias amounts to $N_e\mu$, the assumption is not
problematic for most genetic markers, where estimates of mutation typically range from
10^{-6} per generation (for allozymes) to $10^{-8} - 10^{-9}$ per year (for a single base pair evolving
under neutrality: Brown et al., 1982; Nei, 1987; Sharp and Li, 1989; Li, 1997). However,
biases can be introduced in studies where low migration rates are coupled with rapidly
evolving markers such as microsatellites ($\mu = 10^{-3} - 10^{-6}$ per generation for micro-
satellites; Weber and Wong, 1993; Schug et al., 1997, 1998).

ASSUMPTION 4: THE GENETIC MARKERS ARE NEUTRAL

Because natural selection can act temporally and spatially in a multitude of ways, it is
usually difficult to determine if an individual locus is under selection without a very
specific expectation of its distribution under neutrality. Even so, most researchers do not
worry about being misled by natural selection because multiple loci are used as indepen-
dent replicates of the evolutionary process whenever possible. This permits the estima-
tion of variance among loci by jackknifing or bootstrapping (Weir, 1996), and the
identification of statistical outliers that may be under natural selection (Lewontin and
Krakauer, 1973; Beaumont and Nichols, 1996).

However, species that do not possess obligately sexual reproduction may require
special consideration. In some species of *Daphnia*, temporal fluctuations in allozyme
frequencies seem to reflect fluctuating selection on the underlying clones (Lynch, 1987).
Alternatively, because hatching cues can differ among *Daphnia* clones, gene frequen-
cies may change over time as hatching from the sediments continues (Schwartz and
Hebert, 1987; Wolf and Carvalho, 1989). In either case, long-term studies are needed in
species with clonal reproduction and dormant eggs to fully understand how variation
within and among populations is best translated into an accurate estimate of gene flow.

The widespread use of DNA for population genetics has now made it possible to test
an even more problematic hypothesis: that among-population differences for *all*
allozymes may be maintained by identical forms of natural selection. If this is true,
population genetic studies of these markers could be extremely misleading. Low differ-
entiation among distant populations (suggesting high gene flow) might actually repre-
sent the widespread maintenance of identical alleles at similar frequencies by natural
selection. This hypothesis has apparently been supported by studies where F_{st} differs
greatly between allozymes and other markers for which the neutrality assumption is

more plausible (examples include Karl and Avise, 1992; Pogson et al., 1995; Latta and Mitton, 1997). However, generalizations do not yet seem possible due to methodological problems, conflicting data sets, and additional studies that have found agreement between allozymes and other nuclear genetic markers (e.g., Baruffi et al., 1995; Isabel et al., 1995; Hare et al., 1996; Lehmann et al., 1996; Nadler, 1996; Isabel et al., 1999). Nevertheless, concerns about neutrality suggest that gene flow estimates will be the most robust when multiple types of genetic markers agree.

ASSUMPTION 5: MIGRATION IS NOT DISTANCE DEPENDENT

The island model's name comes from the assumption that migration is not distance dependent (i.e., all populations are equally accessible to all other populations). When migration rates are low and the spatial scale of interest is relatively small, this is likely to be a reasonable approximation. On larger scales, data may be more appropriately analyzed with "hierarchical island models", where nested sets of islands are dictated by the landscape (e.g., Hughes et al., 1995; Schmidt et al., 1995; Bohonak, 1998). Where populations are distributed more continuously, a model of "isolation by distance" may be more realistic (analytic techniques described by Slatkin, 1993). Thomas et al. (1998) used a similar approach for the amphipod *Hyalella azteca*.

ASSUMPTION 6: F_{st} HAS REACHED AN EQUILIBRIUM

As described above, the estimation of gene flow from eq 1 assumes that the system has reached an equilibrium where F_{st} no longer changes over time. Unfortunately, Fig. 1 shows that initial values of F_{st} following a landscape alteration, range expansion, or founder event may take many generations to decay. During the interim, gene flow estimates can be biased in either direction, and the magnitude of the bias can be very large. The possibility that populations rarely have time to reach this equilibrium has led some authors to question the very utility of gene flow estimates (Bossart and Prowell, 1998; Whitlock and McCauley, 1999).

Although our discussion below focuses primarily on large timescales, nonequilibrium conditions may be important *within* years for clonal pond species such as *Daphnia*. An annual succession of clonal genotypes may lead to uncertain results if gene flow estimates are extrapolated from a single sampling date (Lynch, 1987); this problem is exaggerated when the number of clones within a pond is low (Vanoverbeke and De Meester, 1997).

TESTING FOR DEPARTURES FROM EQUILIBRIUM

When the goal of a population genetic study is to infer how often individuals move between populations, it is important to remember that gene flow estimates will be biased if F_{st} has not reached a stable equilibrium (Fig. 1). In the absence of additional information, a particular value of F_{st} may reflect population "history" to an unknown extent, rather than the dispersal of individuals. As a result, genetic studies of pond-dwelling invertebrates have reached a wide variety of conclusions. Genetic homogeneity

has been variously interpreted as the consequence of high gene flow or of recent range expansions (Riddoch et al., 1994; Bohonak, 1999b), while high population differentiation has been thought to indicate low gene flow, extreme founder events, or a metapopulation landscape shaped by local extinctions (Korpelainen, 1986; Weider, 1989; Boileau et al., 1992; Crease et al., 1997; Davies et al., 1997). Because the range of interpretations for any particular value of F_{st} is great, it is imperative that theoretical
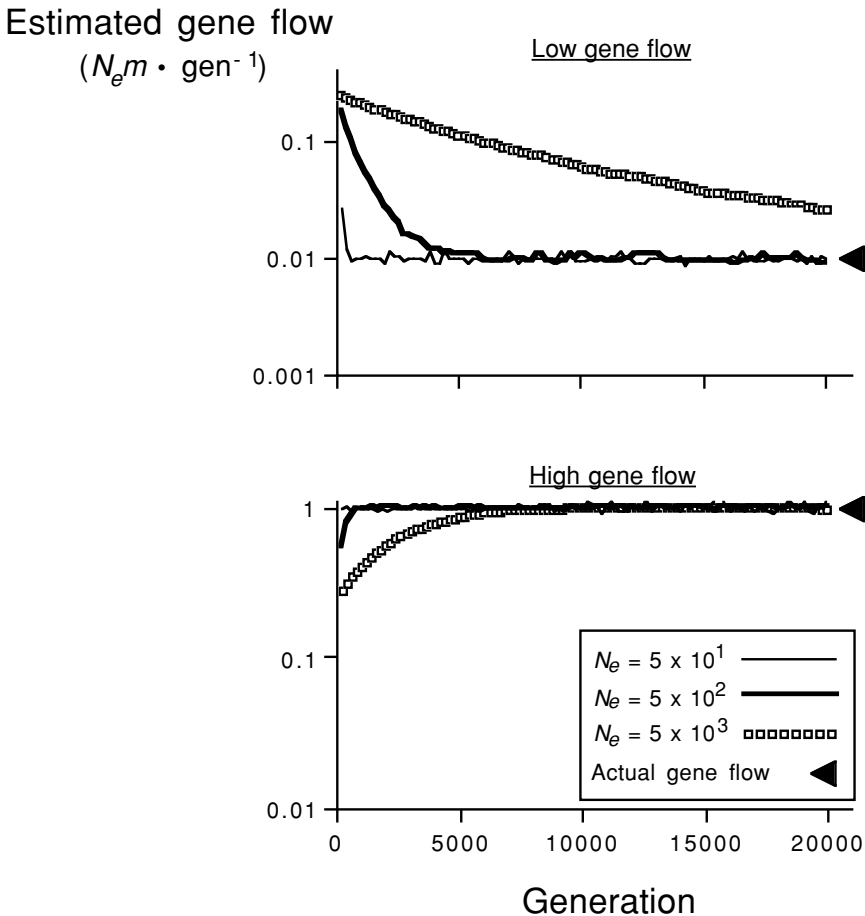

Fig. 1. Effect of nonequilibrium conditions on gene flow estimated using eq 1. Equilibrium is achieved more gradually when gene flow is low and population size is large. Each curve represents the mean of 20 replicates; variance among replicates is due only to random genetic drift and stochasticity of the colonization process (see text). Actual gene flow (average of 1 individual every 100 generations (*top*) or 1 individual each generation (*bottom*)) is indicated with a dark arrowhead. N_e is the number of individuals in each of 100 populations, $\mu = 0$ (no mutations).

expectations and additional information are used to limit these possibilities objectively.

Below, we describe eight ways that departures from equilibrium can be inferred. Using a combination of these approaches, it may be possible to determine where along the trajectories in Fig. 1 any particular species will lie, and in doing so, assess the reliability of the associated gene flow estimate. (Some of these tests will also indicate whether other assumptions associated with eq 1 are being violated.) It is important to note that most of these comparative tests implicitly assume a null hypothesis of equilibrium. Without this assumption, these tests will not be meaningful. In contrast, if equilibrium is assumed to be extremely unlikely a priori, one may decide that eq 1 is too far removed from reality to be useful (Whitlock and McCauley, 1999). It is difficult to imagine a theoretical analysis or comparative test that can conclusively reject a null hypothesis of *non*equilibrium. However, as we discuss below, it may be possible to obtain qualitative information about the movement of individuals among populations even in this case.

TEST 1: ESTIMATE DISPERSAL DIRECTLY

As discussed above, quantitative studies of dispersal in temporary pool invertebrates are relatively rare (e.g., Brendonck and Riddoch, 1999). As a result, dispersal rates are unknown for so many species that there are usually few a priori expectations of dispersal ability before a population genetic study begins. Nonetheless, some invertebrates have proved amenable to both ecological and genetic study, and congruence between dispersal and gene flow estimates is not uncommon (e.g., Nürnberger and Harrison, 1995; Nürnberger, 1996; Bohonak, 1998; Bohonak and Whiteman, 1999). However, it is widely recognized that one should not expect complete agreement between ecologically obtained dispersal estimates and estimates of gene flow for methodological and behavioral reasons (discussed in Slatkin, 1985). For example, local adaptation has been demonstrated for a wide variety of traits in pond-dwelling zooplankton, suggesting that migrants experienced lower fitness after dispersing (see De Meester, 1993, 1996).

TEST 2: ESTIMATE SHORT-TERM GENE FLOW DIRECTLY

Short-term gene flow can be estimated directly by following the movement of rare marker alleles or clonal genotypes (in species without sexual reproduction) among natural populations. This can then be used to evaluate a long-term estimate of $N_e m$ as calculated using eq 1. To date, few researchers have used this approach (e.g., Burton and Swisher, 1984; Grosberg, 1991), probably because of the difficulties involved with screening for rare genotypes, raising organisms in the laboratory, and successfully releasing them back into the environment.

TEST 3: COMPARE SPECIES WITH DIFFERENT DISPERSAL ABILITIES

Just as it is possible to compare short- and long-term estimates of gene flow, one might also wish to compare long-term estimates of gene flow and dispersal. One way to obtain a "long-term" estimate of dispersal is to rank dispersal ability across species based on life history and morphological, developmental, or habitat-related characteris-

tics. If the species are otherwise comparable in terms of behavior, demography, and phylogenetic relatedness, these rankings should provide a conservative way to evaluate the contribution of dispersal to F_{ST}. As long as the ranking criteria are constant over the lifetime of the species, they are qualitatively different than short-term ecological estimates of dispersal. Boileau et al. (1992) utilized this approach for 15 species of freshwater invertebrates in Canada that included Collembola, Ostracoda, Cladocera, Turbellaria, Copepoda, and Anostraca. Rankings of dispersal were inferred from adult dispersal tendencies and attributes of each species' eggs. Boileau et al. (1992) found no correlation between dispersal ranking and estimated gene flow, concluding that the gene flow estimates were inaccurate for these taxa in this region.

Bohonak (1999a) reviewed correlations between F_{ST} and dispersal ability in 27 groups of animals (333 total species). Groups of animals tended to be more taxonomically restricted than those compared by Boileau et al. (1992), and the criteria for ranking dispersal more conservative. In all but one case, the correlation was in the expected direction (higher dispersal rankings led to lower estimates of F_{ST}), with an average rank correlation of –0.72. Thus, when there is dispersal variation within a taxonomically restricted group, species assumed to be more vagile tend to possess lower estimates of F_{ST} and higher estimates of $N_e m$. While this does not imply that gene flow estimates are always accurate, in the absence of an alternative hypothesis, it does indicate that at least *some* information about dispersal ability is usually contained in F_{ST} (see also Bunn and Hughes, 1997; Peterson and Denno, 1998a,b).

TEST 4: ASSAY MARKERS WITH DIFFERENT MUTATION RATES OR EFFECTIVE POPULATION SIZES

Comparing patterns of differentiation among different types of markers may be the best way to determine the relative contributions of migration, mutation, genetic drift, and natural selection. To test the specific hypothesis that gene flow estimates are biased by departures from equilibrium, contrasts can be made between markers with different mutation rates (e.g., microsatellites vs. allozymes) or effective population sizes (nuclear vs. mitochondrial genes). This is because the time required to reach equilibrium decreases as μ increases and N_e decreases (see below). Although these comparisons can result in estimates of population structure that are largely congruent (Lehmann et al., 1996; Besansky et al., 1997), complex interpretations are sometimes inevitable (Burton and Lee, 1994). For example, Crease et al. (1997) interpreted allozyme and mtDNA variation in four species of *Daphnia* in the context of dispersal ability and habitat-specific metapopulation structure.

TEST 5: COMPARE SPECIES OR POPULATIONS IN QUALITATIVELY DIFFERENT LANDSCAPES

It is usually impossible to assay populations repeatedly over a timescale long enough to determine if population differentiation and gene flow estimates are stable (but see Lynch, 1987; Weider et al., 1997; Hairston et al., 1999). As an approximation, F_{ST} can be compared among species that are similar ecologically but are distributed across areas

with different histories. For example, species of fairy shrimp from northern Canada, alpine habitats in Colorado (USA), and vernal pools in California (USA) and Southern Africa show remarkably similar estimates of gene flow despite the unique history of each region (Bohonak, 1998). However, the work of Avise and colleagues (e.g., Avise, 1992) shows that the opposite can also be true. Species with different dispersal abilities and life histories may nonetheless retain common patterns of population structure from a shared geologic history. In these cases, estimates of F_{ST} on large geographic scales would contain information about population history as well as the movement of individuals.

Population "connectivity" can translate into differences in F_{ST} and $N_e m$ as well. Thomas et al. (1998) showed that genetic divergence among *Hyalella* amphipods increases in more xeric landscapes. Similarly, Verspoor (1994) found $F_{ST} = 0.38$ among non-anadromous populations of Atlantic salmon vs. $F_{ST} = 0.03$ among anadromous populations (see also De Meester, 1996; Bohonak, 1999a). In general, the absence of correlations between genetic differentiation and landscape age or connectivity would be consistent with departures from equilibrium and biased estimates of $N_e m$.

TEST 6: LOOK FOR ISOLATION BY DISTANCE

When dispersal is limited, differentiation among population pairs will increase with the geographic distance between them. In these cases, the island model fails to approximate migration patterns satisfactorily. Slatkin and colleagues (Slatkin and Maddison, 1990; Slatkin, 1993) have suggested that when a spatially explicit "stepping-stone" model of migration can be assumed, a pattern of "isolation by distance" is expected at equilibrium. For a stepping-stone model, the genetic distance among all *pairs* of populations is plotted as a function of geographic distance. (Although a variety of genetic distances are used to make plots such as these, Slatkin suggests using $\hat{M}$, an analog of $N_e m$ from eq 1.) A linear slope in plots of log ($\hat{M}$) vs. log (geographic distance) for all pairs of populations is consistent with genetic equilibrium. Peterson and Denno (1998a) have found that isolation by distance is common in insects, suggesting that equilibrium is widespread. For freshwater invertebrates, isolation by distance has been found in Anostraca (Davies et al., 1997; Bohonak, 1998) and Cladocera (Vanoverbeke and De Meester, 1997).

TEST 7: UTILIZE ADDITIONAL PHYLOGENETIC INFORMATION

Data collected at the DNA level contain additional information not utilized in standard frequency-based estimates of F_{ST}. In contrast to simply treating alleles categorically (such as θ: Weir and Cockerham, 1984), estimators such as N_{ST} (Lynch and Crease, 1990), Φ_{ST} (Excoffier et al., 1992), and R_{ST} (Slatkin, 1995) incorporate information about the genealogical or mutational distances among alleles. Empirical studies comparing distance-weighted and distance-free estimates of F_{ST} are now accumulating (e.g., Chenowith et al., 1998; Davies et al., 1999). Most of these have utilized the flexible AMOVA framework of Excoffier et al. (1992) which can accommodate any matrix of pairwise allelic distances. Slatkin and Maddison (1989) have also devised a means for inferring information about gene flow directly from gene genealogies.

Theoretical studies contrasting the properties of these statistics (and the resulting gene flow estimates) have concentrated primarily on equilibrium analyses. However, information regarding departures from equilibrium might also be gleaned from contrasting statistics such as θ and Φ_{ST}. The distances among alleles are expected to be informative when populations have been stable for long periods of time, but misleading if recent invasions have occurred from a diversity of sources (see also Barton and Wilson, 1995; Templeton, 1998). Thus, very different gene flow estimates from distance-weighted and distance-free statistics might suggest dramatic differences between historical and contemporary species ranges or patterns of gene flow.

TEST 8: DETERMINE THEORETICAL EXPECTATIONS

For simplicity, we consider here the theoretical expectations for the simple island model following a widespread colonization event. For this model, the equilibrium value of F_{ST} and the rate at which populations will approach equilibrium depend on five factors:

a.   the effective population size N_e;
b.   the rate of gene flow m;
c.   the mutation rate μ;
d.   the initial state of the populations;
e.   the amount of time since that initial state.

Additional factors such as demographic changes, very few populations, and extinctions might also be considered in more complex models.

The theoretical trajectory of F_{ST} over time is relatively easy to calculate for Wright's island model using a spreadsheet or computer program:

$$F_{ST}^{\,t+1} = \left(\frac{1}{2N_e} + F_{ST}^{\,t}\left(1 - \frac{1}{2N_e}\right) \right)\left(1 - m\right)^2\left(1 - \mu\right)^2 \qquad (2)$$

(Wright, 1969; Latter, 1973; Hartl and Clark, 1989; μ is unimportant if $\ll m$.) Using these formulas, one can determine the expected time to equilibrium for any particular set of parameters. Boileau et al. (1992) used this approach to argue for persistent nonequilibrium conditions in arctic pond zooplankton. For high values of N_e (e.g., 10^6 individuals), the colonization of ponds by very few individuals creates high initial values of F_{ST} that take thousands or tens of thousands of generations to equilibrate. Because the species studied by Boileau et al. are found in ponds less than 3000 years old, actual gene flow could be much higher than N_em as estimated from eq 1.

HYPOTHESIS TESTING

Sometimes clear alternative hypotheses are available regarding processes such as drift, gene flow, departures from equilibrium, and the characteristics of recent colonizations (e.g., Hairston et al., 1999). In these cases, hypothesis testing is possible when assumptions about parameter values such as N_e, N_em, and μ can be made. However, in contrast to

iterations of eq 2, an approach that utilizes computer-simulated populations is flexible enough to permit complex invasion dynamics. The likelihood of drift-gene flow equilibrium or of different colonization scenarios (e.g., single vs. multiple invasions) can be ascertained by comparing simulated values of F_{st} to actual values (see Bohonak and Roderick, in press).

The approach can be applied to the data of Bohonak (1998) to determine if genetic equilibrium is feasible in the fairy shrimp *Branchinecta coloradensis*, sampled from an area of the Rocky Mountains (USA) glaciated approximately 10,000 years ago. Allozymes have been used to estimate $F_{st} = 0.12$ among ponds within valleys ($\approx$ 100 m or less between pairs of ponds) and $F_{st} = 0.71$ on a regional scale (among valleys $\approx$ 4–11 km apart). Assuming an island model at equilibrium, these correspond to $N_e m = 1.8$ and 0.1, respectively. Several lines of evidence seem to indicate that estimates of gene flow are accurate within valleys (Bohonak, 1998; Bohonak and Whiteman, 1999). These include (1) agreement with ecologically and experimentally obtained estimates of dispersal, (2) agreement with estimates of gene flow from other fairy shrimp species in areas of different geologic ages, and (3) a pattern of isolation by distance (F_{st} within valleys $< F_{st}$ among valleys).

To test the hypothesis that differentiation *among* valleys is in equilibrium, we simulated the evolutionary trajectory of hypothetical valleys containing *B. coloradensis* for 12,000 generations. These simulations were conducted with the computer program ESP (Bohonak and Roderick, 2000) and assumed the following:

a. The regional species range of *B. coloradensis* was modeled as (1) 25 valleys, each with $N_e = 2 \times 10^3$ individuals, or (2) 50 valleys, $N_e = 1 \times 10^4$ individuals per valley. These values represent an area within which the island model of migration is a satisfactory approximation. (A diapausing egg that disperses outside of its valley will land in any other valley in this range with approximately equal probability.) Although exhaustive surveys of *B. coloradensis* have not been conducted in this region, the lower value reflects our best estimate of $\approx$ 500 km^2 based on the data currently available (Nelson, 1971; Bohonak, Wissinger, and Brown, unpubl. data), and the upper value is an estimate of maximum regional range. The qualitative results of other values for species range can be inferred from these two cases (see below).

b. Initial differentiation among valleys was created by randomly sampling colonists from a hypothetical ancestral pool (described in Bohonak and Roderick, 2000). For both species range models (25 or 50 valleys), two types of colonizations were simulated. In the first, extreme population bottlenecks led to initial F_{st} values of approximately 0.85. The second scenario involved a large range expansion without appreciable bottlenecks, so that $F_{st} (t = 0) \approx 0.05$.

c. Following colonization, island model migration occurred among valleys at a rate of $N_e m = 0.10$ per generation (as estimated in Bohonak, 1998). Thus, we are determining if this particular level of gene flow could lead to genetic equilibrium on a relevant timescale. (Equilibrium will be achieved more quickly if actual migration rates are higher, and less quickly if they are lower.) This is not a test of equilibrium for the

actual populations, nor does it determine if $N_em = 0.10$ is accurate. It is a test of whether $N_em = 0.10$ *could* be accurate soon after a range expansion. In light of additional geologic or demographic information, additional hypotheses could be tested.

d. Migration *within* valleys was assumed to be very high, so that within-valley differentiation was not considered (i.e., we assume that within-valley F_{st} is close to 0). Because F_{st} within valleys is 0.059 – 0.165 (95% confidence intervals (CI) from bootstrapping over loci; Bohonak, 1998), the model presented here should only be considered a first approximation. Future analyses could incorporate additional population structure within valleys.

e. To evaluate simulation results, one generation was assumed to be equivalent to one year in *B. coloradensis* (Bohonak, 1998; Bohonak and Whiteman, 1999). Mutation rates were set at 1×10^6 / allele / generation.

One thousand simulations were conducted for 12,000 generations for each of the four range size/colonization scenario combinations described above. F_{st} was calculated every 1000 generations using the estimator θ (Weir and Cockerham, 1984) by sampling 109 randomly chosen individuals from each of 3 populations at 5 independent loci. (Sample sizes and number of loci parallel those of Bohonak, 1998.) From these estimates, a median and 95% CI were calculated for F_{st} and for gene flow estimated using eq 1. Results are shown in Fig. 2.

For the smaller value of regional species range (25 valleys, $N_e = 2000$ individuals within valleys), gene flow estimates approach equilibrium relatively quickly. The extreme bottleneck scenario produces values of F_{st} that are close to those currently observed, so departures from equilibrium would not be a concern in this case. When initial F_{st} values are very low, the median gene flow estimate is accurate to within an order of magnitude in less than 1000 generations. By 9000–12,000 generations, population differentiation is nearly stabilized and the bias in estimates of N_em is minimal. In contrast, 50 valleys of $N_e = 1 \times 10^4$ would take appreciably longer than 12,000 generations to reach equilibrium (Fig. 2), although gene flow estimates are accurate to within an order of magnitude by generation 5000.

The size of the confidence intervals reflects the accuracy which can be expected from sampling only three valleys and five loci in these scenarios. More extensive field surveys and the isolation of additional polymorphic loci would reduce variance, although conventional wisdom states that one should only expect gene flow estimates to be accurate to within 50% even under ideal conditions (e.g., Slatkin and Barton, 1989).

Thus, our conclusions for *B. coloradensis* are qualitatively similar to those of Boileau et al. (1992) for other pond-dwelling invertebrates. In temporary pond species with relatively large species ranges and large numbers of breeding adults, gene flow estimates from allozyme data may be biased. In these cases, more rapidly sorting or more rapidly mutating genetic markers would provide less biased estimates of how often individuals disperse among valleys.
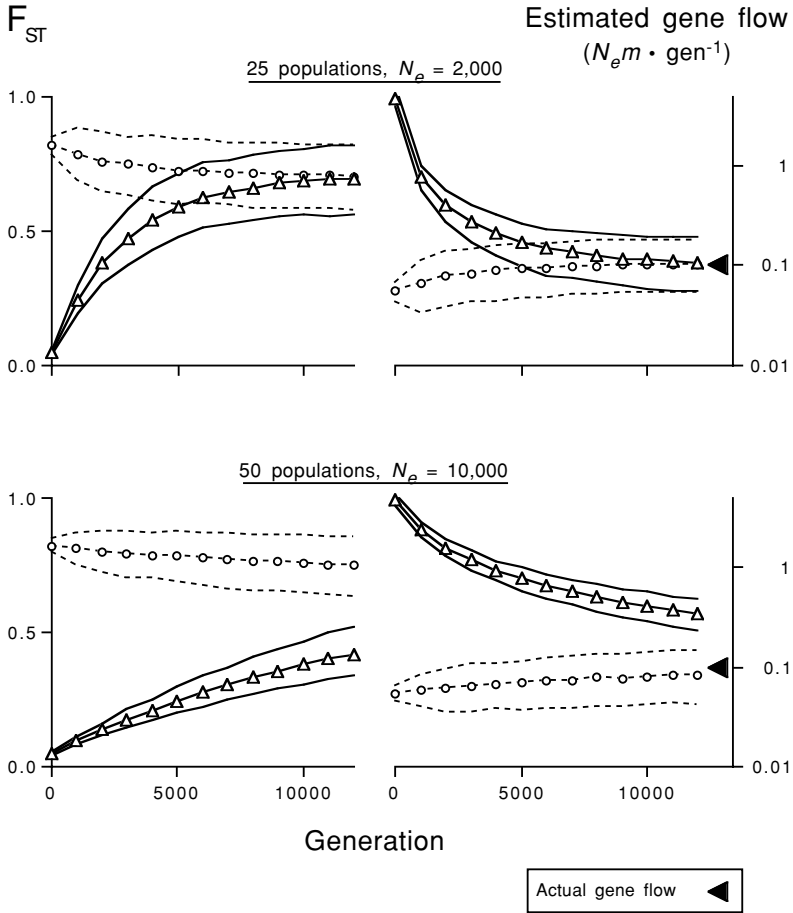
Fig. 2. Estimates of F_{st} and $N_e m$ among valleys from computer simulations. Median values (circles or triangles) and 95% confidence intervals from 1000 replicates are shown for colonization scenarios that began with low differentiation (solid lines) or high differentiation (dashed lines). Actual gene flow estimate from Bohonak (1998) is indicated with a dark arrowhead. See text for details.

IMPORTANCE OF THE ECOLOGICAL THEATER

It is clear that interpretations of population genetic data cannot be made in an ecological vacuum. The extent to which populations differ is only one type of data from which information regarding the movement of individuals may be inferred. Ecological studies will provide other critically needed pieces of information, such as estimates of N_e and generation time. The nature of the landscape is also critical to a valid interpretation of F_{st}. Thus, an enumeration of the number of locally interacting populations, their perma-

nence, and their arrangement in space will prove invaluable. Due to the prevalence of long-lived egg banks in freshwater invertebrates (Hairston, 1996; Hairston and Cáceres, 1996), the frequency of extinctions may play a less important role in differentiating populations. However, extinction and recolonization can dominate habitats and species without dormant propagule reservoirs (e.g., Nürnberger and Harrison, 1995). In these cases, parameters such as the average number of colonists and the population extinction rate would be helpful in interpreting F_{st} and the accuracy of $N_e m$ estimates. Paradoxically, one often turns to gene flow estimates only when these types of data are difficult to obtain directly.

Each of these variables can be visualized as an axis in multidimensional parameter space. Within this parameter space, there are many regions which could produce any particular value of F_{st}. Behavioral, demographic, and historical information can be used to eliminate regions that are biologically unrealistic. Gene flow estimates in any particular study have the potential to be accurate if the regions that remain produce observed values of F_{st} on timescales comparable to the age of the populations. Figure 2 demonstrates that even information from simple field surveys can help determine if genetic equilibrium is feasible, and provide the data necessary for the construction of more detailed models.

THE UTILITY OF GENE FLOW ESTIMATES

Studies of gene flow and genetic population structure have applications in many areas of ecology and evolutionary biology. The genetic independence of populations provides a basis for making decisions in resource management and conservation (Frankham, 1993; Nielsen, 1995; Avise and Hamrick, 1996). The evolutionary potential of a species is a function of its population structure; thus, an understanding of gene flow provides insight for processes such as local adaptation and speciation (Chevillon et al., 1995; Feder et al., 1997). Finally, gene flow estimates from neutral genetic markers can provide a null model for the study of important morphological and behavioral traits (Spitze, 1993; Gillespie and Oxford, 1998). Methodological and theoretical advancements in population genetics over the past 30 years have, to a large degree, reflected the diversity of these applications. Current population genetic studies generally seek to determine the specific processes that maintain or break down population differentiation, rather than simply determining *whether* populations differ genetically. Quantifying gene flow is a central part of this goal.

CONCLUSIONS AND FUTURE DIRECTIONS

We take a decidedly optimistic view regarding the information that genetic data can provide in natural populations. The theoretical literature on F_{st} is extensive (although generally fragmented), and gene flow has been estimated in many pond-dwelling invertebrates on a variety of spatial scales. The six assumptions outlined here are (or should be) well understood (Bohonak et al., 1998; Bossart and Prowell, 1998). Based on

arguments such as those of Boileau et al. (1992), for temporary pond organisms, the equilibrium assumption may be the most important. What has been lacking thus far is a simple enumeration of how departures from genetic equilibrium can be detected in any particular system. The eight tests outlined here are general enough that several of them could be applied to almost any species or habitat to produce a comprehensive interpretation of population structure. In the future, increased computational power, flexibility in analytic techniques, and theoretical advancement are likely to increase the importance of hypothesis testing for empirical population genetics.

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