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Beyond F_{ST}: Analysis of population genetic data for conservation

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Abstract

Both the ability to generate DNA data and the variety of analytical methods for conservation genetics are expanding at an ever-increasing pace. Analytical approaches are now possible that were unthinkable even five years ago due to limitations in computational power or the availability of DNA data, and this has vastly expanded the accuracy and types of information that may be gained from population genetic data. Here we provide a guide to recently developed methods for population genetic analysis, including identification of population structure, quantification of gene flow, and inference of demographic history. We cover both allele-frequency and sequence-based approaches, with a special focus on methods relevant to conservation genetic applications. Although classical population genetic approaches such as F_{ST} (and its derivatives) have carried the field thus far, newer, more powerful, methods can infer much more from the data, rely on fewer assumptions, and are appropriate for conservation genetic management when precise estimates are needed.

Background

The estimation of metapopulation structure and gene flow was one of the first applications of population genetics, and despite spectacular advances in the types and amount of genetic data now available, many methods still used today are based on theoretical foundations built more than half a century ago (Fisher 1930; Wright 1931). These methods have provided population geneticists with the tools to analyze their data and robust, meaningful ways in which to interpret data from natural populations (Weir and Cockerham 1984; Slatkin and Barton 1989; Neigel 2002). However, it is now widely recognized that the idealized models of population structure, migration, demographics, and evolution on which these methods are based are far from realistic and are unlikely to occur in nature (Whitlock and McCauley 1999). This is particularly true in conservation genetic assessments because most populations and species of conservation concern are small and/or have recently declined in size, experienced fragmentation, or otherwise been perturbed. These are exactly the kind of demographic situations that can bias F_{ST}-based estimates of migration (or other approaches that assume mutation-drift equilibrium; Whitlock and McCauley 1999; Kinnison et al. 2002). Despite the limitations, Wright's FST (1951) has remained the standard parameter used to describe the amount of differentiation among pre-defined subpopulations, and from this, to estimate migration rates (Neigel 2002, Weir and Hill 2002). F_{ST}-based approaches are well understood and easily applied, and many investigators rely on these relative measures of genetic variance as the primary descriptors of population genetic structure (e.g., Sites et al. 1999; Larson et al. 2002; Courtois et al. 2003; Saint-Laurent et al. 2003). Thus, in the situations where results have the most practical importance – guiding conservation efforts and identifying genetically distinct management units, evolutionarily significant units, or species boundaries – traditional population genetic analytical methods may be at their worst, and are less-than-optimal at best.

Several authors have provided reviews of the use of F_{ST} in the literature, but have drawn contradictory conclusions about its continued utility. Considering primarily allozyme data, Slatkin and Barton (1989) used simulations to show that F_{ST} was the best method then available not only for describing population subdivision, but also as a basis for estimating gene flow, *Nm*, via Wright's (1951) equation $F_{ST} = 1/(4Nm + 1)$. Slatkin and Barton (1989) noted, however, that although F_{ST} can be applied to DNA data, it cannot use all the information in the data and should be superseded by more sophisticated approaches. Bossart and Prowell (1998) concurred, and called for advances in statistical analysis to match advances in the laboratory, stating, "The challenge now is to develop and implement analytical methods that keep pace with technology and allow more precise estimates of gene flow in contemporary time."

Here we argue that, while F_{ST} will continue to be used as a comparative benchmark in population genetic studies and as a basic descriptor of population structure (Neigel 2002), advances in data collection and statistical analysis are beginning to meet Bossart and Prowell's challenge and have made it possible to infer far more about the historical and current demographics of natural populations than can be done using traditional approaches (Beerli 1998; Beerli and Felsenstein 1999, 2001; Arbogast et al. 2002). In particular, though computationally intensive, coalescent-based analyses can estimate several parameters simultaneously, and are not based on summary statistics but instead determine the overall set of parameters that best describe the data. In addition to an estimate of overall population structure or average divergence comparable to F_{ST}, this parameter set can include estimates of: the number of genetically definable subpopulations in the sample; the number of genetically distinct clusters that have been sampled; differentiation estimates for each pair of populations; asymmetrical pairwise gene flow estimates; the relative effects of isolation and migration; current and historical effective population sizes; and the demographic history of the populations. These approaches are also better able to differentiate among alternative explanations for a given genetic signal than are simple summary differentiation statistics. For example, simple values of F_{ST} cannot distinguish between a situation of high migration between populations with a long divergence time, and one of a relatively recent shared history but no ongoing gene flow. Recently developed methods, on the other hand, have the potential to provide information on the importance of current migration relative to historical associations among populations (Nielsen and Wakeley 2001), as well as demographic information on population history, growth, and variability (e.g. Wakeley 1996; Beaumont 1999; Beerli and Felsenstein 2001).

Maximum likelihood and Bayesian inference approaches (see Box 1) have led to advances in phylogenetic systematics (Lewis 2001), and these same methods are expanding the analytical power available to population and conservation geneticists (Shoemaker et al. 1999). However, although the literature on alternative and coalescent-based estimators of population structure and demographic history is developing rapidly, many of the methods may be inaccessible to conservation biologists attempting to incorporate genetic data into a conservation and management program for an endangered species. In addition, these methods do come with their own limitations, including (1) computational power, which is steadily increasing, (2) user friendliness, which is steadily decreasing, (3) varying assumptions of general levels of diversity, equilibrium, recombination, levels of gene flow, etc. (detailed below by method), and (4) the need for large data sets to simultaneously estimate multiple parameters with accuracy. Furthermore, as in phylogenetic systematics, careless use of complex methods can lead to problems (e.g., over-sensitivity to Bayesian priors, lack of testing for convergence, etc.; Huelsenbeck et al. 2002). Thus users must be aware of potential problems with a chosen method and how they apply to particular data sets. Our goal here is therefore to describe the types of information that can be gained through new analytical approaches to population genetic data (both allele-frequency or sequence based; see Sunnucks 2000 for an overview of DNA marker choice), provide an overview of their use, and direct readers to additional references and methods relevant to particular situations.

Changing genetic data, changing analyses

From its inception, the estimation of F_{ST} has been adapted to each successive form of genetic data

Box 1.

While traditional estimators in population genetics could be calculated using simple analytical calculations, modern population genetic analyses rely heavily on computer power. This is especially true for methods based on MAXIMUM LIKELIHOOD or BAYESIAN INFERENCE.

The concept of LIKELIHOOD was first developed by R.A. Fisher (1925) to distinguish it from the concept of probability. We can define a probability model $P(D|H)$ as the probability P of obtaining the data D given the hypothesis H, according to a probability model. Whereas in likelihood, we have $L(H|D)$, the likelihood L, of the hypothesis H, given the data D and a specific model which is proportional to $P(D|H)$. In other words, with probability the data are variable with a constant hypothesis, while in likelihood the hypothesis is variable and the data are constant (Edwards 1992).

BAYESIAN INFERENCE is described by Bayes' rule as

$$P(H|D) = \frac{P(D|H) \times P(H)}{P(D)},$$

where the prior probability of the hypothesis $P(H)$ is combined with the sampling distribution $P(D|H)$ and conditioned on the known data $P(D)$ to yield a posterior probability of the hypothesis given the data (Gelman et al. 2000). While the posterior probability is easy to formulate conceptually, in practice it is all but impossible to calculate analytically in population genetic models. Therefore, posterior probabilities of population genetic parameters for large data sets are typically approximated using MARKOV CHAIN MONTE CARLO (MCMC) simulation (Gilks 1996), a general iterative method for finding likelihood maxima. This has the advantage of allowing sophisticated data analysis to be conducted in far less computational time than would be required for a full likelihood-based analysis.

Edwards, A.W.F. (1992) *Likelihood*. Baltimore: The Johns Hopkins University Press.

Fisher, R.A. (1925) *Statistical Methods for Research Workers*. Edinburgh: Oliver and Boyd.

Gelman, A., J.B. Carlin, H.S. Stern, and D.B. Rubin. (2000) *Baysian Data Analysis*. Boca Raton: Chapman & Hall/CRC.

Gilks, W., S. Richardson, and D. Spiegelhalter. (1996) *Markov Chain Monte Carlo in Practice*. London: Chapman & Hall.

produced (e.g., G_{ST}, multiple alleles, Nei 1973; sequence data, Weir and Cockerham 1984; NST, restriction-site variation, Lynch and Crease 1990, haplotypes, (AMOVA) Excoffier et al. 1992; Slatkin 1993; Holsinger and Mason-Gamer 1996; Software summarized in Schnabel et al. 1998). The increased use of microsatellite markers, in particular, has led to the development of variations on F_{ST} that attempt to account for the high mutation rates of microsatellite alleles by using the stepwise mutation model (SMM) rather than the infinite alleles model (IAM) in their estimation of differentiation (R_{ST}, Slatkin 1995, $(\delta\mu)^2$, Goldstein et al. 1995, Φ_{ST} Michalakis and Excoffier 1996; ρ_{ST} Rousset 1996; Zhivotovsky 1999). However, microsatellites do not always follow a strict SMM (Ortí et al. 1997; Colson and Goldstein 1999; Gardner et al. 2000; Van Oppen et al. 2000), and even when they do (i.e., in simulation studies), RST does not appear to be consistently superior to FST (Gaggiotti et al. 1999; Balloux and Goudet 2002; Balloux and Lugon-Moulin 2002). Thus, there has been considerable controversy over the best method for the analysis of microsatellite allele frequency data, yet all of the above methods share the common conceptual approach of calculating summary statistics based on the variance in allele frequencies within and among populations. In addition, methods for estimating these summary statistics have increased in complexity. For example, the program HICKORY (Holsinger et al. 2002) estimates F_{ST} from dominant markers such as AFLPs using Bayesian inference, and Weir and Hill (2002) have provided an updated, maximum-likelihood based estimator of Weir and Cockerham's θ (1984). However, despite these sophisticated approaches, the end result is still an FST analog subject to all the limitations of the traditional estimator.

One of the most significant theoretical developments in genetic analysis is the concept of the coalescent, which links demographic history with population genealogy (Kingman 1982; reviewed by Hudson 1990; Nordborg 2001). Approaches based on coalescent methods provide estimates of effective population size and past demography that reflect the evolutionary history of the population rather than the current allele-frequency distribution (Waples 1989; Beerli 1998; Crandall et al. 1999; Williamson and Slatkin 1999; Anderson et al. 2000). Thus, while traditional F_{ST} approaches are still very useful for estimating

current allele distributions within and among populations, coalescent-based methods can use the stochastic reduction in lineage number looking backwards through time to infer the past demographic history of the population based on a model of evolution for the marker being used. By their nature, they rely heavily on computationally intense statistical methods and increasingly larger data sets to make accurate inferences based on the genetic data. Although they will still be limited by our ability to accurately model the processes involved (e.g., microsatellite mutation, as discussed above), the potential benefit is that coalescent-based methods can use more of the information contained in the data to provide additional, more accurate estimates of population parameters compared to methods with less realistic assumptions. A more detailed treatment of coalescent theory is beyond the scope of this paper, but we refer readers to reviews by Hudson (1990), Nordborg (2001), and Stephens (2001).

Beyond population structure: what else can genetic data tell us?

Neigel (1997) described the "standard approach" used in population genetic studies, in which genetic markers are used to estimate F_{ST}, which is in turn used to produce an estimate of migration between populations based on Wright's island model. Although he also considered DNA data and coalescent methods as an alternative to the standard F_{ST} – allozyme approach (Neigel 1997), the field had not advanced enough to provide real alternatives to the traditional methods. In the past seven years, however, new statistical methods have been developed along with an increase in genetic data provided by large sample sizes and DNA markers such as microsatellites, sequences, and most recently, single nucleotide polymorphisms (SNPs; see review by Brumfield et al. 2003). Thus, it is now appropriate to consider alternative approaches for estimating migration rate in natural populations as well as other population parameters that were unapproachable using the F_{ST} – allozyme framework. Below we provide an overview of methods that together provide alternatives at each step of the analytical process, beginning with the definition of populations, and continuing to the estimation of the recent demographic history of each genetically distinct sub-population (Figure 1).

How many populations?

A central problem for conservation genetics is the identification of discrete populations, management units (MUs), and evolutionarily significant units (ESUs) (Moritz 1994; Crandall et al. 2000). This problem can be especially acute when a species is more or less continuously distributed (Diniz-Filho and Telles 2002). Unfortunately, traditional estimators of population structure rely on the *a priori* definition of populations, and their informativeness will be greatly reduced if these pre-defined populations do not accurately describe the biological reality.

Several recent methods attempt to circumvent this problem by dividing the total sample into "clusters" of individuals, each of which fits some genetic criterion that defines it as a group (Table 1: Pritchard et al. 2000; Dawson and Belkhir 2001). These methods are not coalescent based. Rather, individuals are assigned to groups based on their multi-locus genotypes and the assumption that the markers should be in Hardy–Weinberg and linkage equilibrium within each randomly mating subpopulation or deme. Conceptually, these methods stem from mixed-stock assessment tests (Pella and Milner 1987; Smouse et al. 1990), and are related to the methods of Paetkau et al. (1995) and Rannala and Mountain (1997), which assign individuals of unknown origin to a given sampled population based on the allele frequencies in all sampled populations and that of the test individual (see next Section). In a sample partitioning method, however, the goal is not to assign unknown individuals to known populations, but to divide the total sample of genotypes into an *unknown* number of subpopulations. This is a similar goal to various phylogenetic clustering methods, but the assignment approach differs in its treatment of individual multi-locus genotypes and is thus a complementary method that can provide information despite a lack of phylogenetic structure (Moazami-Goudarzi and Laloe 2002).

The most widely used genotypic clustering method thus far is that implemented in the program STRUCTURE (Pritchard et al. 2000). This Bayesian clustering method takes a sample of

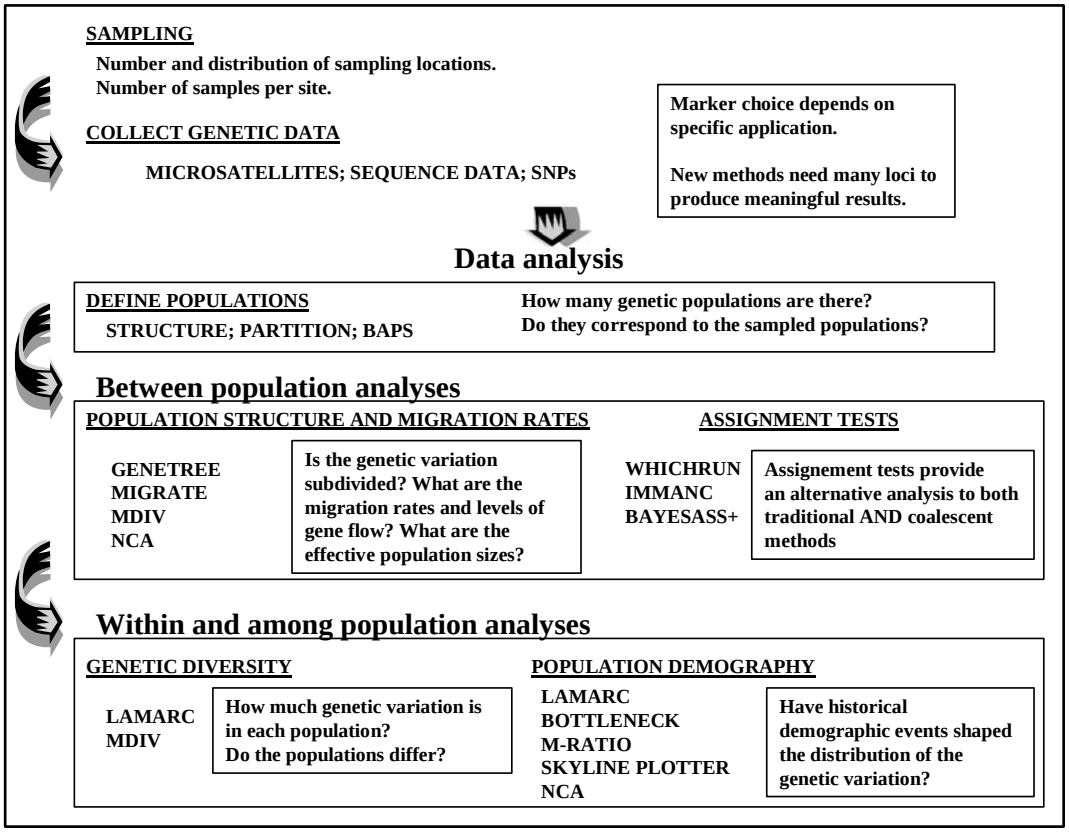

Figure 1: Flowchart of stages in a conservation genetic analysis at which newly developed methods can be applied. At each stage, several analyses are possible depending on the questions being asked. Programs are detailed in the text and in Table 1.

genotypes and uses the assumption of Hardy–Weinberg and linkage equilibrium *within* subpopulations to find (1) the number of populations, k, that best fits the data, and (2) the individual assignments that minimize H–W and linkage disequilibrium in those subpopulations. Thus, with no prior information on population sampling design, STRUCTURE provides an estimate of the number of subpopulations, each of which contains a set of individual genotypes that are in H–W equilibrium. This allows the researcher to let the data define the populations, rather than making best-guess definitions of populations prior to the analysis of genetic structure or using sampling location as a surrogate for the genetic population definition. Furthermore, if all potential source populations have been sampled, the probability output of STRUCTURE can act as a fully Bayesian assignment test for unknown individuals (Manel et al. 2002). Recent advances to the method of Pritchard et al. (2000) include an extension that exploits data on linked markers to infer the source populations contributing to the sample, and an updated version of the software (v2.1; Falush et al. 2003).

A very similar method was developed by Dawson and Belkhir (2001) in their program PARTITION. Like STRUCTURE, PARTITION uses Bayesian inference and a likelihood model to identify population sub-division and assign individuals to populations on the basis of their genotypes at co-dominant marker loci. Also as in STRUCTURE, the basis for the definition of subpopulations in PARTITION is the identification of groups of individuals that conform to Hardy–Weinberg and linkage equilibrium conditions. However, one problem for any assignment test is the presence of admixed individuals in the sample, and an important difference between STRUCTURE and PARTITION is the latter's assumption that all individuals are of pure ancestry; the former allows for the presence of admixed

Table 1. Software available for conservation genetic analyses. See text for details

Program	Data Type	Website	References
How many populations?			
STRUCTURE	Allelic	pritch.bsd.uchicago.edu/software.html	Pritchard et al. (2000)
PARTITION	Allelic	www.univ-montp2.fr/~genetix/partition/partition.htm	Dawson and Belkhir (2001)
BAPS	Allelic	www.rni.helsinki.fi/~jic/bapspage.html	Corander et al. (2003)
Which population?			
DOH	Allelic	www2.biology.ualberta.ca/jbrzusto/Doh.php	Brzustowski (2002)[1] and Paetkau et al. (1995)
WHICHRUN	Allelic	Www.bml.ucdavis.edu/whichrun.htm	Banks and Eichert (2000)
IMMANC	Allelic	www.rannala.org/labpages/software.html	Rannala and Mountain (1997)
NEWHYBRIDS	Allelic	http://ib.berkeley.edu/labs/slatkin/eriq/software/software.htm	Anderson and Thompson (2002)
This population?			
GENECLASS	Allelic	www.montpellier.inra.fr/URLB/index.html	Piry and Cornuet (1999)[1]
Migration and gene flow			
MIGRATE	Sequences, allelic data	evolution.genetics.washington.edu/lamarc/migrate.html	Beerli and Felsenstein (2001)
GENETREE	mtDNA Sequence	http://www.stats.ox.ac.uk/mathgen/software.html	Bahlo and Griffiths (2000)
MDIV	mtDNA Sequence	www.bscb.cornell.edu/Homepages/Rasmus_Nielsen/files.html	Nielsen and Wakeley (2001)
BAYESASS+	Allelic data,SNPs	www.rannala.org/labpages/software.html	Wilson and Rannala (2003)
Growing or declining? inference of past demography			
ARLEQUIN	Sequence, SNPs, microsatellites	http://lgb.unige.ch/arlequin/	Schneider, Roessli, and Excoffier (2000)[1]
FLUCTUATE	Sequence, SNPs, microsatellites	http://evolution.genetics.washington.edu/lamarc/fluctuate.html	Kuhner et al. (1998)
GENIE	Sequences	http://evolve.zoo.ox.ac.uk/software.html	Pybus and Rambaut (2002)
BOTTLENECK		http://www.montpellier.inra.fr/URLB/bottleneck/bottleneck.html	Piry et al. (1999)
M-RATIO	Microsatellites	http://santacruz.nmfs.noaa.gov/staff/carlos_garza/software.html	Garza and Williamson (2001)
BATWING	Linked loci	http://www.maths.abdn.ac.uk/~ijw/downloads/download.htm	Wilson et al. (2003)
MSVAR	Microsatellites	http://sapc34.rdg.ac.uk/~mab/	Beaumont (1999)
GEODIS	Sequences; AFLPs	http://inbio.byu.edu/Faculty/kac/crandall_lab/computer.html	Posada et al. (2000)[1]

[1]Website citation only.

individuals. These individuals of hybrid origin are assigned proportionally to two or more populations (another program, NEWHYBRIDS (Anderson and Thompson 2002), explicitly tests for the presence of admixed individuals in a population and categorizes them (i.e., F_1, F_2, B_1, etc.), a feature that could find applications in many conservation genetic analyses). Because of the inherent uncertainty in the execution of Bayesian programs using Markov Chain Monte Carlo (MCMC) simulation (see Box 1), it is probably advisable to perform multiple runs with a given data set to evaluate the consistency of the results for convergence. Similarly, these methods are relatively untested in terms of their ability to detect population structure or make population assignments when levels of gene flow are relatively high ($F_{ST} < 0.05$; but see Berry et al. 2004), and preliminary results indicate that they may not perform well when many populations (>10) are included in the analysis (IEP, unpublished data). Thus for now we would recommend caution in the analysis of empirical data sets, using multiple runs and a variety of software applications whenever possible, to provide an index of the robustness of these methods and the strength of the signal in the data.

Another approach, taken by Corander et al. (2003) and implemented in the Program BAPS, uses Bayesian assessment to determine the number of genetically distinct populations present in a sample of user-defined populations (*N*p). Like the above methods, BAPS assumes both Hardy–Weinberg and linkage equilibrium, as well as a "reasonably low" mutation rate (Corander et al. 2003). However, BAPS differs from STUCTURE and PARTITION in at least two significant ways. First, it treats each population as a unit rather than considering individuals separately, and uses prior information about the geographic sampling design to inform the analysis. Second, BAPS is based on estimating allele frequencies and determining which of the *populations* have different allele frequencies, rather than partitioning *individuals* into HWE populations based on their multi-locus genotypes. This information is then used to recalculate the allele frequencies in the re-defined populations. The method thus represents a more sophisticated estimator of basic allelic differentiation among populations, comparable to a Fisher's Exact test (Raymond and Rousset 1995). It has the advantage, however, of grouping non-differentiated populations and re-calculating the allele frequencies based on the merging of these populations. The output (allele frequencies in N populations) can then be used in a traditional distance–based analysis, incorporated into an assignment test (see beyond), or used in other population genetic analyses.

Finally, Dupanloup et al. (2002) have developed a method that makes no assumptions about Hardy–Weinberg or linkage equilibrium, and, like BAPS, uses the genetic data to define *groups of populations* that are maximally differentiated from each other. As in BAPS, the number of groups the populations are to be divided into must be defined *a priori*. However, the method of Dupanloup et al. (2002) also considers the spatial relationships among the populations, and thus can identify the locations of barriers to gene flow between groups. This may have significant advantages for conservation geneticists seeking to group populations or MUs into ESUs (Moritz 1994; Crandall et al. 2000).

Assignment tests: which population?

A closely related set of methods use allele frequency data from *known* populations to determine the most likely source of an individual with a given genotype when the actual source of the individual is unknown (Table 1, see also Davies et al. 1999). These approaches are much less computationally intense than the partitioning methods, and have been applied to wildlife forensics (Primmer et al. 2000; Manel et al. 2002) and fisheries stock analysis (Utter and Ryman 1993), as well as the study of migration and dispersal events in natural populations (Waser and Strobeck 1998; Malone et al. 2003). Although crude genetic assignments can be made even with relatively low variability markers provided that fixed differences exist among candidate populations (e.g., mtDNA sequences, Fabiani et al. 2003), hyper-variable marker systems and more sophisticated analytical approaches are needed when the populations in question are less diverged (Paetkau et al. 1995; Rannala and Mountain 1997; Manel et al. 2002).

The most commonly used assignment test was developed by Paetkau et al. (1995) and is implemented in the programs DOH (see Table 1) and

WHICHRUN (Banks and Eichert 2000). The method calculates the likelihood of a given individual's multi-locus genotype originating in each of two or more candidate populations (the allele frequencies of which are estimated from the sample) under the assumptions of Hardy–Weinberg equilibrium and linkage equilibrium (although it seems to be fairly robust to violations of this assumption – Cornuet et al. 1999). It is worth emphasizing that both of these assignment tests are computationally straightforward and easy to interpret, so provide a good complementary analysis that may be used in conjunction with traditional estimators of population structure. Another assignment program IMMANC, developed by Rannala and Mountain (1997), differs in that it uses a Bayesian approach to estimate the population allele frequencies from the sample allele frequencies, while DOH and WHICHRUN assume that the sample allele frequencies represent the population accurately. IMMANC is also capable of not only assigning individuals to their source population, but also of identifying individuals with recent immigrant ancestry (i.e., individuals with immigrant relatives one or more generations removed), even when the source populations are relatively similar.

Cornuet et al. (1999) developed a third assignment test, as well as a modification that statistically *excludes* potential source populations for a given individual rather than attempting to assign the individual to a source population (implemented in GENECLASS, see Table 1). This allows for the situation in which not all potential source populations were sampled, thus asking the question "did this individual come from this population?" rather than "of these sampled populations, which is the most likely source of this individual?" Because the new assignment method of Cornuet et al. (1999) did not perform as well as existing methods, GENECLASS couples the Bayesian assignment method of Rannala and Mountain (1997) with the new exclusion modification. Finally, Manel et al. (2002) compared the partially- Bayesian method of Rannala and Mountain (1997) with the fully Bayesian method of Pritchard et al. (2000). The use of STRUCTURE as an assignment test has the advantage that it considers all individuals and populations simultaneously, rather than testing each individual separately for evidence of migrant genetic signal, and this approach improves the power to detect multiple migrants (Pritchard et al. 2000). However, STRUCTURE assumes that all potential source populations have been sampled, and the lack of this requirement is a significant advantage of the GENECLASS method (Manel et al. 2002).

Alternative estimators of migration rates and divergence time

The migration rates estimated from genetic data represent quantities fundamentally different from estimates derived from physical capture-recapture studies or direct observations. Whereas physical capture or observation methods provide a snapshot of animal movements, as well as ecological information not obtainable from genetic data, indirect genetic estimates of migration rate represent an average of the actual *successful* migration rate among the populations (i.e., migrations that led to reproduction). The most commonly used genetic estimator of migration rate is derived from F_{ST}, and represents the average level of migration among a group of populations, under the assumption of an island model, via the theoretical connection given by the formula of Wright (1931); $F_{ST} \approx 1/(4Nm + 1)$.

Despite the imprecision of this relationship in most meaningful biological settings (and the additional assumptions of equal, constant population sizes and symmetrical migration), this estimator continues to be used both for historical reasons and because of its claim of a direct link between genetic diversity and the calculated migration rate. Even this link, however, has recently been called into question by Whitlock and McCauley (1999), who argue that the unrealistic assumptions upon which this formula is based make the translation of F_{ST} (or R_{ST}) values into estimates of migration rate useless. A similar sentiment was expressed by Beerli (1998), especially in regards to the assumption of symmetrical migration rates.

Coalescent-based methods have been developed to make inferences about populations, including effective population size, growth rate, divergence times, and migration among subpopulations (Beerli and Felsenstein 2001, Nielsen and Wakeley 2001). The last category of estimators in particular promises to provide an alternative to the assumption-laden estimates of Nm derived from

Fst, potentially increasing the accuracy of migration estimates and allowing biologically realistic situations such as asymmetric migration (Beerli 1998). Arbogast et al. (2002) have provided a comprehensive review of divergence time estimation at both phylogenetic and population genetic timescales, and we will not attempt to duplicate their effort here. Instead we focus on alternatives to the Nm estimate traditionally derived from Fst in population genetic analyses.

A variety of coalescent-based methods have now been developed to evaluate migration among populations under different models of population structure and using DNA sequences, microsatellite allele frequencies, or both (Wakeley 1996; Beerli and Felsenstein 1999, 2001; Bahlo and Griffiths 2000; Nielsen and Wakeley 2001; Gaggiotti et al. 2002; Wilson and Rannala 2003). The programs GENETREE (Bahlo and Griffiths 2000), and MDIV (Nielsen and Wakeley 2001) both consider single-locus, non-recombining DNA sequence data (Table 1). Both programs use an infinite-sites model of sequence evolution, which limits their usefulness with highly variable sequences. MDIV (Nielsen and Wakeley 2001) is designed to jointly estimate migration rates (including asymmetric) and divergence times between a pair of populations. This allows the user to distinguish between ongoing migration and the lingering effects of historical association as possible explanations for population similarity, a critical failing of F_{ST}-based estimates of Nm. The method also provides current and historical estimates of population size. Similarly, GENETREE generates a tree and produces coalescent-based maximum likelihood estimates of mutation rate, migration rates, and population growth rates, as well as the time to most recent common ancestor (TMRCA) for the sequences.

A more general program, MIGRATE, was developed by Beerli and Felsenstein (2001) to estimate migration rates and effective sizes of n populations using a maximum likelihood approach. MIGRATE provides results very similar to those of GENETREE, with more flexibility in the underlying model of mutation. MIGRATE is also notable for its ability to utilize different data types, including microsatellite data, rather than only DNA sequences (Table 1). Like MDIV and GENETREE, MIGRATE produces estimates of θ, which provide a coalescent-based inbreeding effective population size for comparison with short-term temporal methods.

All of the above programs are aimed at estimating long-term gene flow among populations, as is traditionally done using the F_{ST}/N_m method. Wilson and Rannala (2003) have developed a non-equilibrium Bayesian method for estimating rates of recent migration among populations. Their method is more akin to the assignment approaches discussed in the previous section in that it uses multi-locus genotypes as probabilistic indicators of source population and thus produces estimates of *recent* migration rather than the long-term gene flow estimates of F_{ST}/N_m or the coalescent-based estimators. It is thus a complementary approach and could be used in conjunction with an estimator of long-term gene flow. Notably, Wilson and Rannala's model is truly non-equilibrium because it doesn't require the loci to be in Hardy–Weinberg equilibrium. Also, rather than simply assigning individuals based on population allele frequencies, BAYESASS+ simultaneously estimates population migration rates, individual migrant ancestries, and the population allele frequencies. This has the important effect of avoiding the bias of including immigrant individuals in the estimation of allele frequencies. BAYESASS+ is appropriate for the analysis of allelic data including allozymes, microsatellites, restriction fragment length polymorphisms (RFLPs), and SNPs.

As a by-product of estimating migration rates, many of the above methods produce an estimate of genetic diversity, $\theta = 4N_e\mu$, where N_e is the inbreeding effective population size and μ is the mutation rate per site per generation. With an estimate of mutation rate for the gene region or regions under consideration, we can solve for the current and/or historical effective population size. Crandall et al. (1999) reviewed a number of earlier genetic estimators of effective population sizes as well as conceptual issues associated with this parameter, but since then many new methods have been developed (Beerli and Felsenstein 2001; Nielsen and Wakeley 2001; Yang 2002; Wall 2003). As is the case with genetic estimates of migration rates, however, indirect genetic estimates of effective population size represent a value fundamentally different from census size estimates derived from either physical studies or from recently developed *direct* genetic methods (Palsbøll et al. 1997; Mills et al. 2000; Pearse et al. 2001).

For example, Turner et al. (2002) found that although N_e estimates from both a temporal change in allele frequency method (short-term variance N_e) and MIGRATE (deep coalescent-based inbreeding Ne) were very similar, they were much lower than the current census size suggested. Similarly, Roman and Palumbi's (2003) use of MIGRATE to estimate θ from mtDNA sequences for several species of North Atlantic whales suggests that historical population sizes were much larger than previously thought. All of these findings illustrate the great potential for making demographic inferences from genetic data when appropriate analyses are applied. However, these methods are in need of further benchmarking through computer simulation in terms of exploring their accuracy, statistical power, and robustness to violations of their assumptions, as well at the type and quantity of data needed. Initial studies suggest that these methods may be problematic under certain circumstances. Abdo et al. (2004) used a coalescent simulation approach to test the accuracy of MIGRATE to estimate genetic diversity (θ), migration rates, and confidence intervals. They ran 1000 simulations for each parameter set of three different levels of genetic diversity by four different migration rates by two different sequence lengths. The results indicated that under these specific conditions, MIGRATE performed well at estimating genetic diversity, but poorly at estimating migration rates and confidence intervals. Clearly more extensive simulations exploring a greater variety of situations, including violations of method assumptions, is critical for a good understanding of the performance of such methods.

Growing or declining? inference of past demography

One of the fundamental questions in conservation genetics is to determine the historical demography of a population. For example, we have a need to distinguish between small populations that naturally have limited genetic variation versus those that have reduced genetic variation due to a recent severe reduction in population size. Conversely, a population may be large in current census size yet small in effective population size due to a past bottleneck (Crandall et al. 1999; Turner et al. 2002). In either case the influence of past demography on current genetic variability can have important management implications in terms of the genetic stability of populations and the potential impact of inbreeding depression on population viability (Vilà et al. 2003). Similarly, in invasive species biology, it is critical to identify the genetic front of population expansion and identify where geographically this expansion is taking place. Recently, population geneticists have addressed the need to partition recurrent evolutionary processes such as gene flow from individual historical events (e.g., past fragmentation, colonization, bottlenecks, or range expansion events). By partitioning out these historical events, one can obtain better and more realistic measures of the potential for ongoing gene flow in the populations of interest. Emerson et al. (2001) provided an excellent review of many of the recently developed methods to identify past demographic events. Here we will highlight the more commonly used approaches to provide an introduction to the various methods available, as well as reviewing additional methods developed since that review (Table 1).

DNA sequences

Rogers and Harpending (1992) showed that under an infinite-sites model the shape of the distribution of the number of observed differences between pairs of DNA sequences can be used to infer population expansion or contraction events. The theoretical expectation is that an episode of population growth produces a distinctive "wave" in the distribution of pairwise genetic distances (the mismatch pair distribution, Harpending and Rogers 2000). Bottlenecks are predicted to generate a similar wave, but with elevated upper tail probabilities. However, the pattern of the mismatch distribution following a population contraction or growth event can be affected by levels of gene flow (high versus low). High gene flow causes most coalescent events to occur around the expansion/contraction event, resulting in a unimodal mismatch distribution, while low gene flow causes coalescent events to occur early in the genealogy resulting in a bimodal distribution (Ray et al. 2003). Therefore, we advise caution in the interpretation of results, given the multiple possible explanations. This method has recently been expanded to include a finite-sites model of evolution accommodating rate heterogeneity coupled with a bootstrap procedure to define confi-

dence intervals around the estimated parameters (Schneider and Excoffier 1999), and can be implemented in the software package ARLEQUIN (Table 1). Considering single-locus sequence data, Nielsen (2001) pointed out that neutrality tests such as Tajima's *D*-test (Tajima 1989) are actually unable to distinguish between the signal generated by selection on the locus and that of a past bottleneck in the population, and thus could function equally well as a bottleneck test, especially if multiple loci revealed the same pattern. Conversely, the action of selection on DNA loci should not be ignored in conservation genetic studies, especially if only a single locus is used (Ford 2002).

Coalescent-based approaches have also been developed that incorporate a population growth parameter in the model of population dynamics such that the exponential growth rate can be modeled as $N_e(t) = N_e(0)e^{-rt}$, where r is the estimated growth rate of the population and is positive for expanding populations and negative for declining populations, $N_e(t)$ is the effective population size at time t in the past, and $N_e(0)$ is the initial or current effective population size. Theoretically these methods can not only determine if the population has been through a recent period of growth or decline, but also the rate of growth or decline. Kuhner et al. (1995, 1998) have developed a method, implemented in the program FLUCTUATE, that samples effectively across alternative (reasonable) gene genealogies for non-recombining sequences. However, analytical and simulation results have shown that the estimate of growth rate is biased upwards when a finite number of individuals is sampled (Kuhner et al. 1998), a problem shared with a similar, earlier approach by Griffiths and Tavaré (1994).

One drawback of the above parametric model for population growth is the assumption of exponential growth (or decline). There is typically no *a priori* reason to make this assumption for a given population, especially in conservation genetics applications. To avoid this assumption, Strimmer and Pybus (2001), (see also Nee et al. 1995; Pybus et al. 2000) developed a nonparametric approach they called the generalized skyline plot. The skyline plot assumes a single coalescent history with rate correlation among different branches. It then defines internode intervals along this history and allows the population size to vary among intervals. The generalized skyline plot, then, is the plot of the estimated population sizes at these internode intervals, which are assumed to correspond to a constant time interval. Although this approach relaxes the strict assumption of the exponential growth for the population, it assumes a single evolutionary history instead of performing the importance sampling of FLUCTUATE. Rooney et al. (2001) have used this general approach to examine population dynamics and historical population sizes in Bowhead whales. The generalized skyline plot is implemented in the software package GENIE (Pybus and Rambaut 2002).

Microsatellite data

Because they are highly variable and can be scored as multi-allelic Mendelian markers, microsatellite loci are especially useful for the inference of recent demographic events, including detection of human-induced impacts on populations. Thus, a variety of methods have been developed specifically for microsatellite data to identify historical demographic events. Due to the unique mutational dynamics of microsatellites, approaches that use them to detect demographic events rely on models of the evolution of microsatellite alleles (Kimmel et al. 1998; Beaumont 1999). Of particular note are methods that use microsatellite data to detect evidence of past reductions in population size. Like the assignment tests discussed previously, these methods are not coalescent based, but rely on the high polymorphism of microsatellite loci and expectations of equilibrium allele or genotype frequencies and distributions to detect the influence of past demographic events on standing genetic variation. The simple nature of these tests makes them computationally straightforward, and they have been successfully used on a number of empirical data sets (e.g., Spencer et al. 2000; Beebee and Rowe 2001; Waldick et al. 2002). The program BOTTLENECK (Piry et al. 1999) detects past population reductions by testing for a transient ($\sim$0.2–4.0 N_e generations) excess in measured heterozygosity compared with the heterozygosity expected at equilibrium (Cornuet and Luikart 1996; Luikart and Cornuet 1998). This excess in heterozygosity is generated because rare alleles are quickly lost due to drift during a bottleneck, but they contribute little to the expected heterozygosity. This same property – loss of rare alleles – also

led to the development of an entirely different test by Garza and Williamson (M-RATIO, 2001). Here the test statistic is the ratio between the number of alleles present at a given microsatellite locus, k, and the range in allele sizes in base pairs, r, so that $M = k/r$. This ratio will be reduced in a population that has suffered a bottleneck; the rare alleles are lost by drift more often than common alleles during a population size reduction, but unless all rare alleles are at the ends of the allele size distribution the range of alleles sizes will not be affected (Garza and Williamson 2001). Because these two methods utilize different properties of microsatellite loci, and thus different effects of a bottleneck on variability at those loci, concordance in their results would provide increased confidence in the evidence for past demographic reduction in a population.

As discussed above for DNA sequences, coalescent-based methods have been developed to infer historical demographic parameters from microsatellite data. BATWING (Wilson et al. 2003), a direct descendent of MICSAT (Wilson and Balding 1998), uses MCMC to conduct a Bayesian estimation of mutation rate, effective population size, growth rates, times of population splitting events, and time since most recent common ancestor (TMRCA). BATWING can consider either microsatellite or SNP data sets, but is limited in that it *assumes complete linkage* among loci (e.g., loci identified on the Y-chromosome), as well as no migration between subpopulations after an initial split.

Beaumont (1999) developed a coalescent-based method to detect demographic changes using the more typical, unlinked, microsatellite loci used in population genetic studies, and suggested that it and other methods like it would lead to a revolution in population genetic analysis. As predicted, it has since been successfully applied to empirical data sets as well as extended to estimate more parameters and make fewer assumptions (Storz and Beaumont 2002). For example, Storz et al. (2002) were able to document a historical population decline followed by a stable population size throughout recent times in savannah baboons, a result consistent with findings using the program BOTTLENECK, but extending beyond the range of non-coalescent demographic approaches into deeper population history.

Finally, attempts have been made to reconstruct complex histories of population introduction, colonization, and expansion over very recent timescales (<100 years). Although making reliable inferences in complex, non-equilibrium situations remains challenging, these sophisticated Bayesian methods have found some success in reconstructing colonization histories (Estoup et al. 2001; Estoup and Clegg 2003). They are currently limited by computational power, but improvements in parameter estimation efficiency through the use of MCMC simulation may provide increased speed and precision, allowing a greater range of demographic histories to be recovered (Estoup et al. 2001).

Phylogeography and landscape genetics

Avise et al. (1987) introduced the word phylogeography to encompass the idea of comparing phylogenetic relationships within a geographic context, and this idea subsequently developed into an entire sub-discipline in population genetics (Avise 2000). The principal aim of phylogeography is to examine population structure in the context of geographic distributions of organisms. Early efforts, however, lacked an explicit framework in which to evaluate the causes of phylogeographic patterns, and the first statistical approach developed to overlay genealogical information on geographic distribution and partition historical demographic events from recurring population structure was the nested clade analysis (NCA), introduced by Templeton et al. (1995). The claimed advantage of the NCA approach over earlier methods is that it partitions historical events both temporally and spatially. Thus, instead of testing for a single demographic event, NCA is presumably able to infer multiple events such as past fragmentation, colonization, or range expansion, and these events can occur at the same time in different regions of the species distribution or at different time points in the evolutionary history. To accomplish this, NCA uses the reconstructed gene genealogy to define a hierarchically nested statistical design, and then overlays sample location information on that nested design to identify the relationships between the geographic distances and the genetic distances. These relationships are then compared with predictions from

population genetic theory to infer historical events impacting the current distribution of genetic variation. Emerson et al. (2001: Box 3, Table 1) suggest that NCA makes fewer assumptions about the data than other methods designed to detect demographic events. The underlying genealogy used by NCA is estimated with the software TCS (Clement et al. 2000); NCA is then performed using the program GEODIS (Posada et al. 2000).

Recently, Knowles and Maddison (2002) have criticized NCA on the grounds that (1) it fails to account for the stochastic nature of demographic histories and, (2) in contrast to the methods previously discussed, NCA lacks a statistical assessment of support for one inferred demographic history over another. Although NCA has been shown to perform well at identifying a specific demographic event in empirical data sets with well-established *a priori* expectations (range expansion in species now inhabiting previously glaciated areas, Templeton 1998), Knowles and Maddison (2002) question the validity of other inferences made using NCA, and call for a merging of statistically robust methods of parameter estimation with the broad-reaching inference approach of NCA. However, Knowles and Maddison (2002) offer no such method. Templeton (2004) has defended NCA on both theoretical grounds (both supporting the method and critiquing the study by Knowles and Maddison) and empirical grounds (demonstrating the utility of this approach using a large number of empirical data sets with presumed historical events).

An alternative to the broad-reaching phylogeography approaches is landscape genetics (Manel et al. 2003). These developing methods focus on finer spatial scales compared to phylogenetic approaches, and use a variety of statistical methods to detect population genetic discontinuities and correlate them with geographic features (reviewed by Manel et al. 2003). Thus, the best evaluations of population genetic data to answer conservation questions may come from the combination of phylogeographic and landscape genetic approaches with the statistical approaches described in previous sections to best define the population structure and demographic history of a species (Masta et al. 2003).

Concerns

To be sure, these newly developed methods come with a variety of concerns as well. Most of these methods have not been thoroughly evaluated in terms of robustness to violations of assumptions, accuracy of inferences, and relative efficiencies with respect to amounts of data and computational time (e.g., Abdo et al. 2004; Berry et al. 2004). With the ever increasing number of parameters, one should question whether or not the amount of data are sufficient to produce accurate estimates. Any method is dependent upon reasonable sampling of both organisms and genes, and it is unclear how robust the inferences from these methods might be to nonrandom or poor sampling. Studies are now underway to evaluate some of these methods using computer simulation.

Prospects

We have provided an overview of aspects of statistical population genetic analysis relevant to conservation genetics. However, we do not claim it is completely comprehensive, nor have we begun to cover all areas of statistical genetics that have seen recent advances based on maximum likelihood and/or Bayesian inference methods (e.g., relatedness (Milligan 2003), parentage calculations (Emery et al. 2001; reviewed by Jones and Ardren 2003), hybridization/admixture analyses (Anderson and Thompson 2002; Randi and Lucchini 2002)). Furthermore, the development and testing of new methods is proceeding at a pace that will quickly render this review out-of-date. Finally, as noted above, many of the methods we describe are in their infancy and remain to be fully evaluated, so comparisons of existing methods should be done to allow direct evaluation of their performance (e.g., see the April 2004 special issue of *Molecular Ecology*). Thus, our hope is that this review will provide a useful starting point and guide to methods of data analysis suitable for conservation genetics, and even more importantly, that it will stimulate further comparative testing and analysis of these methods relative to conservation genetic data. Bayesian approaches, especially those coupled with faster summary statistics (e.g., Ray et al. 2003), seem to hold great promise for the future of conservation genetic data analysis

(Beaumont and Rannala 2004). Thus, to achieve a better understanding of the population dynamics and population histories of endangered flora and fauna, the most thorough analyses will incorporate a variety of approaches with complementary assumptions and strengths to effectively move beyond a simple estimate of F_{ST}. Future work needed to fully realize the utility of many of the methods discussed herein includes:

- Faster implementation with more user-friendly software. The usability of available programs varies considerably, and for large data sets, run time can be a significant barrier (as is the case for many phylogenetic programs). In addition, programs that require advanced knowledge and an inordinate amount of user effort in order to produce reliable results are unlikely to be widely embraced by empirical geneticists. Thus, improving software usability is essential. A related problem is that as the number of available programs increases, so too does the number of input file formats. This has led to the need for programs to convert input files for a variety of programs (e.g., MSA, Dieringer et al. 2003; CONVERT, Glaubitz 2004).
- Consideration of sample size issues. Several authors have noted that increasing the accuracy of demographic growth, population size, and migration rate estimates for all of these coalescent-based methods is dependent on generating data from more *loci* rather than more *individuals* per population (Beerli 1998; Kuhner et al. 1998; Wilson and Rannala 2003). However, for any empirical study using these methods it will still be important to assess the minimum number of samples per population needed to provide reasonable power to differentiate populations based on allele frequencies, particularly with highly variable markers.
- Relaxation of the central assumptions that are typically violated by real data (e.g., mutation models for microsatellites, equilibrium genetics, large population size, infinite alleles, etc.). Many newer methods relax at least some of these assumptions, and Wilson and Rannala (2003) have provided the first estimator of migration rate that explicitly does not require Hardy–Weinberg equilibrium. Equally important is evaluation of the ability of these methods to perform when actual population divergence levels are very low, as in the case of high gene-flow species (e.g., Waples 1998; Berry et al. 2004).
- The interpretation of genetic data in conservation contexts needs to be carefully evaluated as our use of finer resolution genetic markers and sophisticated analysis increases (this applies to estimates of F_{ST} as well as results from newer analyses). For example, there is little consensus on the relative risks of inbreeding versus outbreeding depression among populations with different divergence levels (Friar et al. 2001), an important issue when considering the management implications of a conservation genetic study. Similarly, what constitutes ''significant'' genetic differences among populations is an open question that has important management implications.

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