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# Detecting Wildlife Poaching: Identifying the Origin of Individuals with Bayesian Assignment Tests and Multilocus Genotypes

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**Abstract:** *Illegal harvesting is a serious threat to the persistence of many plant and animal taxa. The combination of highly polymorphic DNA markers and new statistical methods called "assignment tests" can potentially help detect and thereby reduce poaching. Assignment tests can identify the population of origin of individuals if populations are genetically differentiated. We evaluated the usefulness of two assignment tests to wildlife forensics by applying them to large empirical (microsatellite DNA) data sets from 10 species. We also conducted computer simulations to assess the influence of genetic polymorphism (heterozygosity) and population differentiation ( $F_{ST}$ ) on the performance of the tests. The fully Bayesian assignment test of Pritchard et al. (2000) performed better than the partially Bayesian exclusion test of Cornuet et al. (1999), but the fully Bayesian method requires the assumption that the true population of origin was sampled. The median percentage of individuals correctly assigned for the 10 empirical data sets was 61% and 36% for the assignment and exclusion tests, respectively. Both the empirical and simulated data sets suggest that nearly all individuals can be assigned with high statistical certainty (99.9%) for two highly differentiated populations ( $F_{ST} \approx 0.15-0.2$ ) when 10 loci ( $H > 0.6$ ) and samples of 30-50 individuals are used per population. We recommend using both tests when the true population of origin might not have been sampled in the data set.*

Detección de la Caza Ilegal de Vida Silvestre: Identificación del Origen de Individuos con Pruebas de Asignación Bayesiana y Genotipos Multilocus

**Resumen:** *La captura ilegal es una amenaza seria para la persistencia de muchos taxones de plantas y animales. La combinación de marcadores altamente polimórficos de ADN y de nuevos métodos estadísticos ("pruebas de asignación") pueden ayudar potencialmente a detectar, y por lo tanto, reducir la caza ilegal. Las pruebas de asignación pueden ayudar a identificar a la población de origen de individuos si las poblaciones están genéticamente diferenciadas. Evaluamos la utilidad de dos pruebas de asignación en aplicaciones forenses de vida silvestre al aplicarlas a una serie grande de datos empíricos (ADN microsatélite) de 10 especies. También llevamos a cabo simulaciones en computadora para evaluar la influencia del polimorfismo genético (heterozigocidad) y de la diferenciación poblacional ( $F_{ST}$ ) en la ejecución de la prueba. La prueba de asignación completamente Bayesiana de Pritchard et al. (2000) dio mejores resultados que la prueba de Cornuet et al. (1999) parcialmente Bayesiana; sin embargo, el método completamente Bayesiano emplea el supuesto de que la población verdadera de origen fue muestreada. La mediana del porcentaje de individuos correctamente asignados por los 10 juegos de datos empíricos fue de 61% y 36% para las pruebas de asignación y exclusión respectivamente. Tanto los juegos de datos empíricos, como los simulados sugieren que casi todos los individuos pueden ser asignados con un alto nivel de certeza estadística (99.9%) a dos poblaciones altamente diferenciadas ( $F_{ST} \approx 0.15-0.2$ ) cuando se emplean 10 loci ( $H < 0.6$ ) y muestras de 30-50 individuos por población. Recomendamos el uso de ambas pruebas cuando la población verdadera puede no haber sido muestreada en las bases de datos.*

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## Introduction

Approximately 10–20% of all vertebrate and plant species are at risk of extinction over the next few decades (World Conservation Union 1996, 1997, 2000). Overexploitation by humans threatens about one-third of the endangered species of vertebrates (Primack 1998). One of the most serious threats to the survival of plant and animal populations is poaching, which can be defined as any act that intentionally contravenes the laws and regulations established to protect renewable wildlife resources (Muth & Bowe 1998). It refers to the illegal harvest, sale, purchase, transport, possession, and use of wildlife (or their parts). Recent evidence indicates that the problem is more widespread than is often perceived (Muth 1998; Muth & Bowe 1998).

Wildlife and their products represent the third greatest illegal traffic after drugs and arms (Robert 2000). For example, it is estimated that each year 25–30 thousand primates are used for trade, in biomedical research, as pets, and in zoos, circuses, and private collections. This number is estimated to be 2–5 million (zoos and pets) for birds, 2–3 million for reptiles, 500–600 million for ornamental fish, 9–10 million for orchids, and 7–8 million for cacti (Hemley 1994). It is not known how much of this use or trade might be illegal.

The most important international treaty prohibiting the trade of endangered species is the Convention on International Trade in Endangered Species (CITES), established in 1973. Unfortunately it is difficult to enforce such treaties and antipoaching laws.

Molecular forensic methods are being used to help enforce wildlife conservation laws by identifying the species of origin from tissue mtDNA (Palumbi & Cipriano 1998; Roman & Bowen 2000) or nuclear DNA (Palumbi & Cipriano 1998) and by matching individuals to tissues through DNA fingerprinting (Taberlet & Luikart 1999; Waits et al. 2000). Genetic methods have also been used to determine the geographic origin of a population sample (e.g., a commercial fisheries catch; Seeb et al. 1990) and paternity (or maternity) in forensic cases. A fifth use of molecular methods is becoming feasible for criminal investigation. It is now possible to determine the population of origin (i.e., birthplace) of a single individual (or products or tissues from an individual) by using hypervariable molecular markers (e.g., microsatellites) and new statistical approaches called assignment tests. These tests exploit the vast information in multilocus genotypes. Identifying the population or geographic region of origin of poached individuals could facilitate the detection of illegal harvesting and trade routes (Primmer et al. 2000) and thereby help focus efforts to reduce or eliminate poaching (Primmer et al. 2000).

There has been much in the literature about the potential usefulness of assignment tests in population studies (Waser & Strobeck 1998; Davies et al. 1999; Luikart & England 1999). The first tests were based on frequency statistics and

calculated the probability of drawing a single multilocus genotype from each potential source by using the observed allele frequencies at each locus in each source population (Paetkau et al. 1995). In response to the growing use of Bayesian statistics in population genetics, Rannala and Mountain (1997) developed a partially Bayesian assignment test that uses a Bayesian approach to estimate population allele frequencies and a frequentist approach to compute the statistical significance of individual assignments.

Cornuet et al. (1999) used simulations to compare the performance of the frequency and partial Bayesian methods and showed that the Bayesian approach provides slightly higher assignment accuracy. Cornuet et al. (1999) also extended assignment methods to include a statistical significance test (based on Monte Carlo simulations) that does not require the assumption that the true population of origin has been sampled (i.e., an exclusion test). Recently, Pritchard et al. (2000) developed a fully Bayesian method that provides a coherent framework for incorporating the inherent uncertainty of parameter estimation into the inference procedure. This method yields a posterior probability value that can be interpreted directly as the probability of origin of each individual from each population sampled (assuming the true population of origin has been sampled).

With a growing range of approaches available for assigning individuals to a population, it is becoming difficult for population managers and conservation geneticists to choose and use appropriate methods. Studies that compare the performance of methods and provide guidelines are needed. There has been surprisingly little mention in the literature of the usefulness of assignment tests for combating poaching.

Our objectives were to (1) explain and illustrate how Bayesian assignment tests or exclusion tests (along with existing DNA markers) can be of immediate use in wildlife forensic cases (e.g., poaching), (2) determine the assignment accuracy of the Bayesian tests when applied to published microsatellite DNA data sets from mammals, insects, fishes, and other taxa, and (3) provide rough guidelines for the application of the tests. We used 10 large empirical data sets containing populations that are isolated from one another. Real data from populations with no current gene flow are necessary for an empirical evaluation of the usefulness of assignment methods in wildlife forensics cases. We also used simulated data sets to assess the influence of heterozygosity and population differentiation ( $F_{ST}$ ) on the assignment accuracy of the tests.

## Methods

### Data Sets

For the 10 published empirical data sets, no recent migration has been reported between the populations except

perhaps for some of the Brown trout populations (*Salmo trutta*; A. Estoup, personal communication). It is possible, however, that there are immigrants in some populations from other populations that are not in the data set (Table 1). Presence of immigrants is often impossible to know for real data. Nonetheless, it is important to evaluate the performance of assignment tests on real data. Each data set contained 8–22 polymorphic microsatellites and 4–15 populations (Table 1). To assess the performance of assignment methods on each data set, we assigned or excluded individuals to or from populations with two published software programs described below. Because the geographic origin or population of origin of individuals was known, we could calculate a percentage of correctly assigned individuals.

To assess the influence of the mean gene diversity (i.e., expected heterozygosity,  $H$ ) and  $F_{ST}$  on assignment performance, we simulated data using an individual-based model with Mendelian inheritance. Populations were initiated by randomly sampling alleles from independent loci. The simulations modeled a Fisher-Wright population with separate males and females (following Luikart et al. 1999a). Each simulated data set contained two isolated populations with 50 breeding individuals ( $N_e = 50$ ; half were females) and 10 loci with five alleles per locus in the initial generation. The allele frequencies in the initial generation were either at equal frequencies ( $H = 0.8$ ) or with frequencies of 0.2, 0.59, 0.1, 0.07, 0.04 ( $H = 0.6$ ). The  $F_{ST}$  and  $H$  were calculated at the end of each simulation, which lasted approximately 5–40 generations to achieve a range of  $F_{ST}$  values (0.05–0.3). Mutation was not included in the model because it is unlikely to have had much effect over only a few generations in two small populations (Luikart et al. 1999a). Our model simulates small, fragmenting populations, as exist in many threatened species. For each simulation with a given final  $F_{ST}$  and  $H$  value, we calculated the percentage of individuals assigned correctly. To assess the effect of  $H$  on assignment accuracy, we fit polynomial regression lines through the points for populations with  $H$  between 0.6–0.8 and 0.3–0.5.

### Statistical analysis

We computed the mean (and range) of all pairwise  $F_{ST}$  values and the mean gene diversity (heterozygosity) for each multipopulation data set using GENEPOP (version 3.2; Raymond & Rousset 1995) and Pop100gene (available from <http://www.ensam.inra.fr/urlob/pop100gene/pop100gene.html>).

In light of previous studies (Cornuet et al. 1999) that found the partial Bayesian method slightly more accurate than the frequency-based methods (e.g., Paetkau et al. 1995), we evaluated only the exclusion test of Cornuet et al. (1999) based on the partial Bayesian method (Rannala & Mountain 1997) and the fully Bayesian assign-

ment method recently introduced by Pritchard et al. (2000). Both methods assume Hardy-Weinberg proportions within populations and independence and linkage equilibrium between loci. These assumptions are necessary to use the observed allele frequencies to compute the probability of origin of a genotype in a population (Waser & Strobeck 1998). Deviations from Hardy-Weinberg could bias estimates of the probability of origin. However, Cornuet et al. (1999) conducted simulations of populations deviating slightly from Hardy-Weinberg equilibrium (deficit of heterozygotes) and found little effect on assignment-test performance.

The partial Bayesian method we used is in fact an exclusion method derived from the assignment method developed by Rannala and Mountain (1997). The principle of this method has already been thoroughly described (Cornuet et al. 1999; Davis et al. 1999). In summary, assignment methods (e.g., Rannala & Mountain 1997) use allele frequencies from a population sample to compute the likelihood of a genotype occurring in the population. They compare the likelihood of the genotype in question to a distribution of likelihoods of simulated genotypes for each candidate population. The genotypes are generated by Monte Carlo simulations of 10,000 independent individuals for each candidate population. If the individual genotype likelihood is outside the tail of the distribution (e.g., 0.001), we can exclude the population as the origin of the individual. If we exclude all but one population, we then assign that population as the origin. This method (and only this one) does not require the assumption that the true population of origin has been sampled, because it does not compare populations but rather treats each one separately. We computed the statistical certainty of assignment or exclusion for each individual by using the exclusion simulation method in the GeneClass software (available from [www.ensam.inra.fr/urlob](http://www.ensam.inra.fr/urlob)).

The fully Bayesian approach (Pritchard et al. 2000) computes the posterior probability that an individual multilocus genotype originates from a population among the sampled populations and compares the individual's posterior probability of originating from a population to a chosen threshold (e.g., 0.999). For example, if the estimated probabilities of origin for the three populations are 0.9999, 0.0001, and 0.0000, then we can assign the individual to the first population and exclude the other populations ( $T > 0.999$ ). But if the probabilities for the three populations are 0.990, 0.000, and 0.010, then we cannot assign the individual because no population has a probability of origin greater than the chosen threshold ( $T = 0.999$ ) introduced for forensic applications. This method uses prior information about the possible population structure. We considered the locations of origin of all individuals to be known (except the one to be assigned) with an equal probability of being in each population, and assumed no admixture (gene flow) among candidate populations. To

**Table 1.** Characteristics of the 10 empirical data sets used in this study, including the number of loci, populations, and individuals in each population.

Species	Loci	Populations	Individuals
Bighorn sheep ( <i>Ovis canadensis</i> ) Forbes & Hogg 1999	8	7	British Columbia: 31 Nevada: 31 Montana, Bison Range: 22 Alberta, Ram Mountain: 30 Alberta, Sheep River: 77 Montana, Sun River: 53 Colorado, Tarryall: 32
Taurine cattle ( <i>Bos taurus</i> ) MacHugh et al. 1997	19	7	Angus: 33 Carolais: 36 Frisian: 40 Hereford: 34 Jersey: 34 Simmental: 36 Kerry: 41
Fish ( <i>Salvelinus alpinus</i> ) Primmer et al. 1999	8	15	population 1: 7 population 2: 20 population 3: 21 population 4: 22 population 5: 20 population 6: 20 population 7: 11 population 8: 15 population 9: 26 population 10: 28 population 11: 27 population 12: 28 population 13: 5 population 14: 16 population 15: 5
Ibex ( <i>Capra ibex</i> ) Maudet et al. 2002	19	6	Switzerland, Albris: 36 Switzerland, Augsmatthorn: 20 Italy, Grand Paradiso: 32 France and Italy, Mercantour: 17 Switzerland, Mount Pleureur: 32 France, Vanoise: 32 Turkey, Anatolian black: 41 Turkey, Angora: 31 Pakistan, Beetal: 34 Italy, Bionda: 32 Egypt, Zaraibi: 32 Italy, Girgantara: 31 Wales, Kildeer: 33 Malaysia, native: 30 Mongolia, native: 31 Spain, Murciana: 31 Switzerland, Saanan: 32 Switzerland, Togenberg: 31 Nigeria, West African dwarf: 34
Goats ( <i>Capra hircus</i> ) Luikart et al. 1999b	22	13	

continued

**Table 1.** (continued)

Species	Loci	Populations	Individuals
<i>Drosophila</i> ( <i>D. Melanogaster</i> ) England 1997	9	11	dif 1a: 36 dif 1b: 36 dif 1c: 36 dif 1d: 36 int 1a: 36 int 1b: 18 int 1c: 18 int 1d: 18 int 1e: 18 int 1f: 18 int 1g: 18
Bumble bees ( <i>Bombus terrestris</i> ) Estoup et al. 1996	8	4	population 1: 20 population 2: 20 population 3: 20 population 4: 22
Brown trout ( <i>Salmo trutta</i> ) Estoup et al. 1998	15*	10	FA1:25 IFA2: 30 FA3: 30 M1: 30 M2: 30 M3: 30 F2: 30 F3: 30 FEP1: 28 WP2: 26
Honey bees ( <i>Apis mellifera</i> ) Estoup et al. 1995	8	6	Sweden: 22 Portugal, North Rif: 28 Maroc, Gharb: 28 Romania: 30 Italy, Sicily: 50 Chacidique: 30 Montana, NCDE: 40
Bears ( <i>Ursus horribilis</i> ) Paetkau et al. 1998	8	5	Wyoming, Yellowstone: 57 Alaska, Izembek: 14 Alaska Kodiak: 30 Sweden, South: 152

\*Includes several allozyme loci.

compute the posterior probability, we used the Structure software (Pritchard et al. 2000) available from [www.pritch.bsd.uchicago.edu](http://www.pritch.bsd.uchicago.edu).

When evaluating both methods, we followed the following five main steps for each data set.

(1) Eight to 22 polymorphic loci were analyzed for 13 or more individuals from each candidate population to be potentially excluded or assigned (only 5 individuals were sampled from two of the fish populations) (Table 1).

(2) Allele frequencies at each locus within each known population were estimated with a Dirichlet distribution (Balding & Nichols 1995; Rannala & Mountain 1997; Cornuet et al. 1999).

(3) The assignment accuracy for each method applied to each data set was calculated with a cross-validation approach, "the leave one out" method (Efron 1983). This means that each individual was removed from the data set, allele frequencies were recomputed, and the in-

dividual was assigned to a population. GeneClass offers this leave-one-out option. For Structure we used a slightly modified version of the software that allowed the leave-one-out procedure to be conducted on each individual in the data set sequentially. This modified version (called Strucassign) is useful for assignment-accuracy evaluation of any data set and is available on request from the author (S. M.).

(4) With Structure, an individual was assigned to a population if its computed probability of originating in the population was higher than a chosen threshold ( $T$ ) introduced for forensic applications (we used  $T = 0.9$ – $0.999$ ). The  $T$  corresponded to a value that could potentially be used in a court case. With GeneClass, a different condition was used: an individual was assigned to a given population if its probability of originating in all other populations was less than a chosen  $p$  value (e.g., 0.001). The performance of each method for each species was assessed as the percentage of individuals correctly assigned (Structure) or excluded from all but the true population of origin (GeneClass).

(5) The error rate was computed as the percentage of individuals excluded from their true population of origin. We considered only this most serious type of error even though other errors have been described (e.g., fail-

ing to exclude some population of non-origin; Cornuet et al. 1999). With Structure and GeneClass, the true population of origin was (erroneously) excluded if the computed probability for the true population was less than a chosen threshold (e.g.,  $T = 0.001$ ) or the  $p$  value (e.g., 0.0001).

## Results

Individuals from the 10 data sets were assigned by the Bayesian method (Pritchard et al. 2000) and by the exclusion-simulation significance test (Cornuet et al. 1999) of the method of Rannala and Mountain (1997). The median percentage of individuals correctly assigned for all data sets was 61% for Structure ( $T = 0.999$ ) and 36% for GeneClass ( $p = 0.001$ ) (Table 2). The percentage of correctly assigned individuals obtained with Structure for each data set ranged from 85% for brown bears (*Ursus horribilis*) to only 28% for the ibex (*Capra ibex*) (Table 2). The accuracy of the GeneClass exclusion method ranged from 75% to 2% for the bears and ibex, respectively. The median error rates for the 10 data sets were 0% for Structure (range: 0–0.341,  $T = 0.001$ ) and 0.76% for the exclusion method (range: 0–2.153,  $p = 0.001$ ) (Table 2).

**Table 2.** Performance of the exclusion simulation test (GeneClass) and Bayesian assignment test (Structure) used to assign individuals to a population of origin.

Species	$F_{ST}^a$	$H_{mean}^a$	GeneClass		Structure	
			individuals correctly assigned (%) <sup>b</sup>	cases where the correct population is excluded (%) ( $p < 0.001$ )	individuals correctly assigned (%) ( $T > 0.999$ )	cases where the correct population is excluded (%) ( $T < 0.001$ )
Bighorn sheep	0.262 (0.056–0.020)	0.574 (0.540–0.640)	64.39	0.719	78.41	0.360
Taurine cattle	0.116 (0.076–0.149)	0.500 (0.473–0.557)	22.13	0.791	62.45	0.000
Fish	0.377 (0.097–0.569)	0.503 (0.358–0.684)	37.37	1.476	39.48	0.000
Ibex	0.164 (.067–0.278)	0.399 (0.309–0.464)	1.77	0.588	27.65	0.000
Goats	0.140 (0.049–0.195)	0.596 (0.328–0.703)	17.23	2.153	60.77	0.239
<i>Drosophila</i>	0.295 (0.124–0.541)	0.430 (0.334–0.550)	51.74	0	65.97	0.000
Bumble bees	0.401 (0.108–0.660)	0.401 (0.122–0.628)	35.37	1.22	60.97	0.000
Brown trout	0.285 (0.114–0.499)	0.289 (0.197–0.426)	17.30	0	45.67	0.000
<i>Apis mellifera</i>	0.341 (0.102–0.564)	0.504 (0.262–0.653)	43.62	1.064	51.06	0.000
Bears	0.324 (0.076–0.149)	0.552 (0.360–0.694)	75.43	0.683	84.982	0.341
Median (SD)			36.37 (21.750)	0.76 (0.620)	60.87 (16.483)	0.00 (0.149)

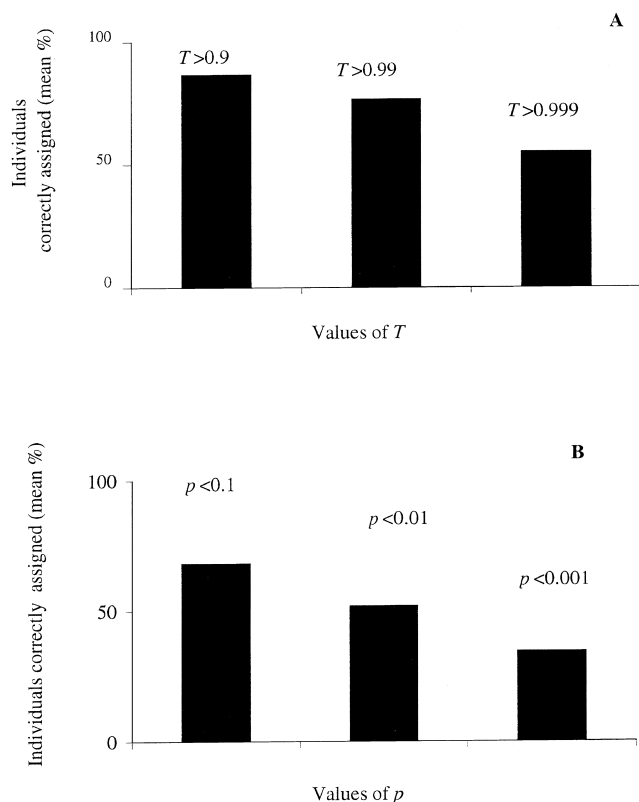
<sup>a</sup>Mean  $F_{ST}$  and mean gene diversity ( $H_{mean}$ ) are given for each data set, with the range for all population pairs in parentheses.

<sup>b</sup>All populations that are not the true population of origin were excluded ( $p < 0.001$ ) but not the true population of origin ( $p > 0.001$ ).

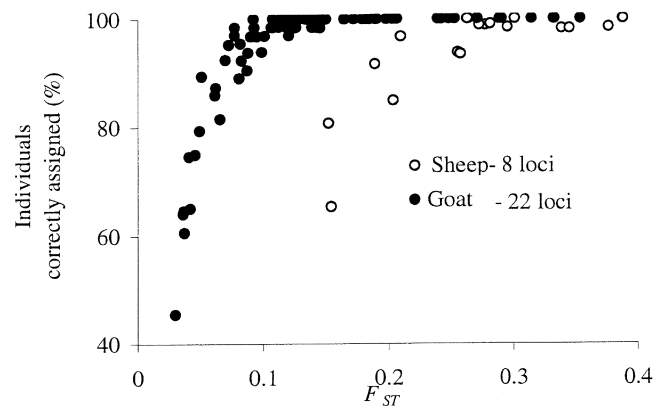
These error rates are interpreted as follows: the true population of origin was excluded for about 0.00% and 0.76%, respectively, of the 2560 individuals across the 10 data sets.

The mean percentage of individuals correctly assigned for all data sets was strongly influenced by the threshold-probability criterion ( $T$  or the  $p$  value) used to assign individuals to a population. Nearly 30% more individuals were correctly assigned when the threshold was only 0.9 instead of 0.999 for the assignment's level of certainty (Fig. 1). For forensic investigations, a high level of assignment certainty (e.g.,  $T > 0.999$ ) is necessary. For most of this study, we used 0.999, which should provide sufficient statistical certainty for many forensic cases.

We assessed the influence of  $F_{ST}$  and the number of loci on assignment accuracy when using only Structure and all pairs of populations from each of two empirical data sets, bighorn sheep (*Ovis canadensis*) and domestic goats (*Capra hircus*). When two populations had an  $F_{ST}$  near 0.1–0.2 (and 8 or 22 loci [ $H \sim 0.6$ ]), 90%–100%



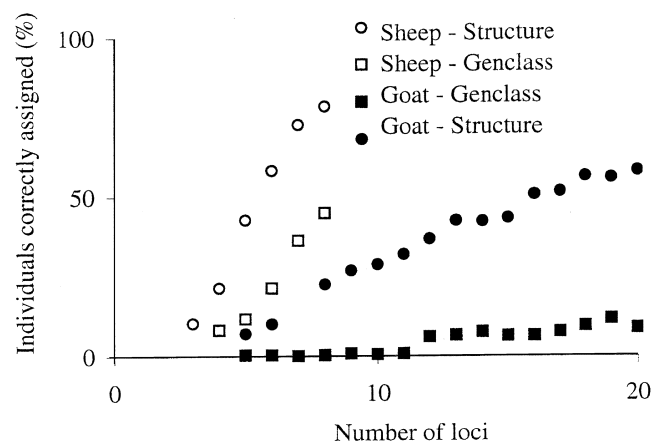
**Figure 1.** Influence of the value of the assignment threshold on the mean percentage of individuals correctly assigned to their population of origin with (a) Structure, the fully Bayesian method, and (b) Geneclass, the exclusion-simulation method of assignment, from all analyzed data sets. Results are shown for different values of the threshold probability ( $T$ ) or  $p$  values used to assign each individual to a population.



**Figure 2.** Relationship between  $F_{ST}$  and the percentage of individuals correctly assigned to their population of origin with Structure, the fully Bayesian assignment method. The threshold level for assignment was  $T > 0.999$  (see Fig. 1). Each point represents one pair of bighorn sheep populations or one pair of goat populations. Data sets are described in Table 1.

of individuals were correctly assigned, each with high certainty ( $T > 0.999$ ) (Fig. 2). When all the populations for each of the two data sets were considered, removing 20% of the loci (with lowest  $F_{ST}$ ) decreased the accuracy of assignment by approximately 20% and 50%, respectively, for the assignment and the exclusion tests (Fig. 3).

To assess the joint influence of  $F_{ST}$  and  $H$ , we used simulated data to estimate the percentage of individuals correctly assigned for different values of  $F_{ST}$  and  $H$ . As



**Figure 3.** Influence of the number of loci on the percentage of individuals correctly assigned to their population of origin with Structure, the fully Bayesian assignment method, and Geneclass, the exclusion-simulation method of assignment. Loci were added from 1 to all loci (8–22 loci), starting with the most heterozygous loci. The threshold level for assignment was  $T > 0.999$  (see Fig. 1).

expected,  $F_{ST}$  strongly influenced the percentage of individuals correctly assigned. There was little difference between loci with high heterozygosity ( $H = 0.6$ – $0.7$ ) and low heterozygosity ( $H = 0.3$ – $0.5$ ) when  $F_{ST}$  was low ( $0.05$ ) (Fig. 4). When  $F_{ST}$  was  $0.2$ , however, nearly 100% of individuals were correctly assigned for loci with high heterozygosity ( $0.6$ – $0.7$ ), whereas only about 80% were correctly assigned for loci with relatively low heterozygosity ( $0.3$ – $0.5$ ) (Fig. 4).

## Discussion

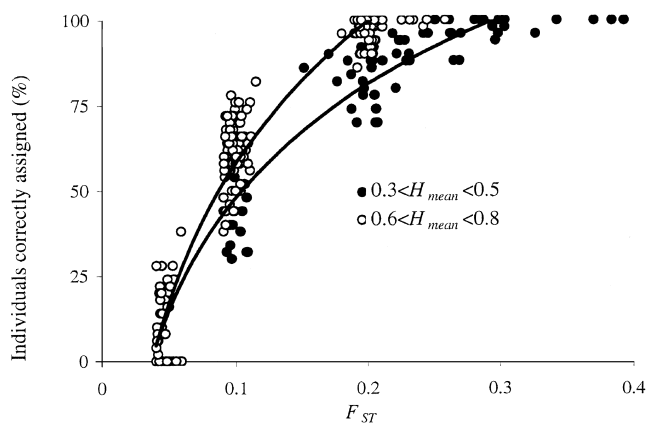
Our most promising result was that 80–100% of individuals were correctly assigned with high certainty ( $T > 0.999$  for each individual) when Structure was used on real data sets and when only two populations with an  $F_{ST}$  of approximately  $0.10$ – $0.20$  were compared. This means the level of certainty of origin was  $>99.9\%$  for 80–100% of the individuals in the empirical data sets. The simulated data roughly agree with the real data (e.g., bighorn sheep, 8 loci; Fig. 2), suggesting that approximately 10 loci ( $H = 0.6$ ) provided nearly 100% assignment accuracy when  $F_{ST}$  was near  $0.2$  for two populations (and when 30–50 individuals were sampled per population). Our results were also roughly consistent with those of Cornuet et al. (1999), who evaluated the exclusion tests through (coalescent) simulations of 10 large, isolated populations ( $N_e = 500$ ) with  $F_{ST}$  near  $0.1$ . The scenario of only two candidate populations of origin will be common in forensic applications. For example, if a putative poacher claims to have obtained an individual from population A, but law enforcement officials believe the individual came

from population B (e.g., a nature reserve), an assignment between the two populations can be conducted (if allele-frequency data exist for both populations).

One striking result is that the assignment method of Structure determined the true population of origin for nearly twice as many individuals (61%,  $T = 0.999$ ) as the exclusion simulation method of GeneClass (36%,  $p = 0.001$ ). This difference was not as surprising, however, when we considered that the two methods involve a different approach for computing the probability of assignment (frequentist vs. Bayesian) and address a somewhat different initial question. For GeneClass, the probability of belonging to each population is computed separately by simulating a frequency distribution of 10,000 genotypes for each population. Thus, the frequentist exclusion test asks the following question: if 10,000 genotypes are randomly sampled from population X, how many times would the genotype in question likely be observed? If the genotype in question is observed less than once in 1000 randomly simulated genotypes, then population X would be excluded as the origin ( $p < 0.001$ ). This is a frequentist approach, because if the experiment were repeated 1000 times, a misassignment by chance alone would be expected less than once in 1000 experiments.

For Structure, the probability that a genotype originates from a population is computed by considering all the sampled populations jointly. The fully Bayesian assignment test computes the posterior probability that the sampled individual is from population X or Y. If  $T > 0.999$ , then the genotype is 1000 times more likely to occur in population X than in Y. This is a Bayesian approach because the probability value is obtained directly (Shoemaker et al. 1999) and prior information can be used. Because the statistical framework and the questions addressed by the two tests are different, it is not easy to directly compare the performance of the two tests.

Although the GeneClass exclusion test was less accurate than the assignment test of Structure, it offers two advantages. First, it does not require the assumption that the true population of origin has been sampled in the data set. This assumption is necessary for Structure because even if the true population has not been sampled, the method might still show that one population is the most likely origin (Cornuet et al. 1999). For example, if in reality there are three populations (A, B, and C) with a true assignment probability of  $0.998999$ ,  $0.001$ , and  $0.000001$ , and only the two last populations (B and C) actually have been sampled, the fully Bayesian method could assign an individual to population B with high probability ( $T > 0.999$ ) because this method compares the relative probabilities (i.e.,  $0.001$  is 1000 times more probable than  $0.000001$ ). But with the exclusion simulation method, both populations B and C would probably be excluded as the true origin ( $p < 0.001$  and  $p <$



**Figure 4.** Relationship between  $F_{ST}$  and the percentage of individuals correctly assigned to their population of origin with the fully Bayesian assignment method of Structure ( $T > 0.999$ ) for different values of  $H$  (mean heterozygosity at the end of simulation). Each point represents two simulated populations.

0.000001). This type of hypothetical scenario requires further investigation.

Second, the exclusion test can be used even if only one population has been sampled. For example, if a putative poacher claims to have obtained an animal (or tissue) from population A, one could (1) use GeneClass to simulate a distribution of 10,000 random multilocus genotypes that are expected to occur in population A (based on the observed allele frequencies) and (2) exclude population A as the origin of the animal if its multilocus genotype lies outside the distribution of the 10,000 simulated genotypes. If the animal's genotype occurred once in the 10,000 simulated genotypes, then its estimated probability of origin from population A would be  $1/10,000$  ( $p = 0.0001$ ).

In an actual example, the exclusion method was used to exclude the possibility that a 5.5-kg salmon originated from the location of a fishing competition, Lake Saimaa in south-east Finland (Primmer et al. 2000). The Structure assignment test cannot be used on data from only one population because it computes the probability of origin by comparing populations, and the sum of the probabilities of origin from each population adds up to one (1.000). This example illustrates an advantage of the exclusion method.

In our study, the relatively low percentage of individuals correctly assigned (61% and 36%) by the two tests may appear disappointing, but this does not mean the tests will never be useful. Our results suggest that in 36–61% of forensics cases with multiple (4–10) candidate populations, the Structure or GeneClass software and available molecular markers would determine the origin of an individual (or tissue or product) with high certainty ( $T > 0.999$ ). In these multipopulation data sets, some of the low assignment accuracy results mainly from one or two population pairs with a low  $F_{ST}$  (e.g., 0.05 for some goat and bighorn sheep populations and 0.06–0.07 for some bear and ibex populations).

If the candidate populations are potentially connected via dispersal, it is probably not worth conducting genetic analyses for purposes of forensic assignment. Forensic applications usually require high certainty of the origin of individuals. Thus, if one cannot be highly certain that dispersal does not occur between the candidate populations, one can seldom be highly certain that an individual harvested in one population did not simply disperse from the other populations. Forensic assignment tests will be most useful in populations from distant geographic locations where populations are completely isolated. This is often the case for species with populations isolated in different mountain ranges or separated by large water bodies.

Another reason for the low assignment performance is that we used a stringent threshold for assignment confidence (e.g., 0.999 for Structure). This threshold was used for each individual assigned to a population. Most

other studies have not used a confidence threshold for each individual assigned (e.g., Paetkau et al. 1995; Blott et al. 1999). For poaching applications (e.g., convicting poachers), a high threshold will be necessary for each individual to be assigned (i.e., 0.999 for Structure).

We assessed the influence of heterozygosity on assignment accuracy. Results of one study suggested that heterozygosity has little influence on assignment performance (Blott et al. 1999), but results from another study suggest that markers with higher heterozygosity provide higher assignment accuracy (Estoup et al. 1998). We found that heterozygosity had an important effect when the  $F_{ST}$  was  $>0.1$ . We also found that the empirical data set with the lowest assignment accuracy (ibex) had the lowest  $H$  (0.49), even though it included many loci (22). Thus, highly polymorphic loci are advisable ( $H = 0.6$ – $0.8$ ) and further study is needed.

### Perspectives and Recommendations

The performance of the methods could be improved by using more loci or loci that are more polymorphic and have a higher  $F_{ST}$ , by sampling more individuals, and perhaps by combining nongenetic methods such as stable isotope analysis (Chamberlain et al. 1997). It may even be possible to use DNA from other species (parasites) for forensic cases. For example, parasites (or plant material) found on illegally obtained animals might have a characteristic genetic signature of a certain population or region of origin. This approach of using parasites (or insects) has been successfully applied in human forensic cases (Amendt et al. 2000).

Now that statistical and molecular tools are available, we recommend that biologists establish large DNA reference collections and large public databases containing allele frequencies from many populations. These databases are necessary for forensic applications that can help conserve endangered species and reduce illegal harvesting. For many species, the necessary markers and data are already available to initiate such large databases.

### Conclusions

The currently available molecular markers and statistical assignment and exclusion software can allow, in some cases, the identification of the population of origin of individuals (or tissues) with high certainty (e.g.,  $T > 0.999$ ). Assignment methods should work reliably at least in cases with few candidate populations that are genetically differentiated, and have no immigration or cryptic substructure. Additional studies are needed on the effects of substructure and immigration from unsampled populations before the tests can be widely used with confidence. For forensics cases, extreme care must be



taken in collecting, labeling, storing, and analyzing tissue samples to avoid human errors. Both the assignment test and exclusion test generally require 10–20 loci with high variability (e.g.,  $H > 0.4$ – $0.6$ ) and moderately high interpopulation differentiation in allele frequencies ( $F_{ST} > 0.1$ – $0.2$ ). Finally, if the true population of origin might not be in the data set, the exclusion test should be conducted along with the assignment test to help avoid assignment errors. Clearly, new assignment methods can help identify poached individuals, the geographic location where poaching occurs, and the trafficking routes of wildlife products. Thus, we are optimistic that these methods can help detect and reduce poaching.

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