

# Can assignment tests measure dispersal?

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## Abstract

Individual-based assignment tests are now standard tools in molecular ecology and have several applications, including the study of dispersal. The measurement of natal dispersal is vital to understanding the ecology of many species, yet the accuracy of assignment tests in situations where natal dispersal is common remains untested in the field. We studied a metapopulation of the grand skink, *Oligosoma grande*, a large territorial lizard from southern New Zealand. Skink populations occur on isolated, regularly spaced rock outcrops and are characterized by frequent interpopulation dispersal. We examined the accuracy of assignment tests at four replicate sites by comparing long-term mark-and-recapture records of natal dispersal with the results of assignment tests based on microsatellite DNA data. Assignment tests correctly identified the natal population of most individuals (65–100%, depending on the method of assignment), even when interpopulation dispersal was common (5–20% dispersers). They also provided similar estimates of the proportions of skinks dispersing to those estimated by the long-term mark-and-recapture data. Fully and partially Bayesian assignment methods were equally accurate but their accuracy depended on the stringency applied, the degree of genetic differentiation between populations, and the number of loci used. In addition, when assignments required high confidence, the method of assignment (fully or partially Bayesian) had a large bearing on the number of individuals that could be assigned. Because assignment tests require significantly less fieldwork than traditional mark-and-recapture approaches (in this study < 3 months vs. > 7 years), they will provide useful dispersal data in many applied and theoretical situations.

*Keywords:* assignment test, dispersal, genetic differentiation, microsatellite, skink

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## Introduction

Most organisms live in patchy environments. Ecologists seeking to understand how species persist in these environments usually estimate four key population parameters: rates of birth, death, emigration and immigration (Hanski 2001). Birth and death rates are typically measured by direct observation, often based on mark-and-recapture data. Emigration and immigration (dispersal) can also be measured by direct observation, but estimation with genetic markers is another important and developing approach (e.g. Peacock & Smith 1997).

Animal dispersal and gene flow have been measured with genetic markers for over 60 years (e.g. Dobzhansky & Wright 1943). These parameters are often estimated indirectly from the spatial distribution of genetic variation among populations under the assumptions of Wright's island model (Wright 1931; Neigel 1997). However, this approach does not distinguish between contemporary and historical gene flow and dispersal, making it difficult to integrate such estimates with the instantaneous rates typically recorded in ecological studies (e.g. birth and death rates). Other limitations of using Wright's island model to measure dispersal are reviewed by Whitlock & McCauley (1999). In contrast, individual-based assignment tests, which assign individuals probabilistically to candidate populations by their multilocus genotype, are widely believed to hold the potential to estimate contemporary rates of gene flow

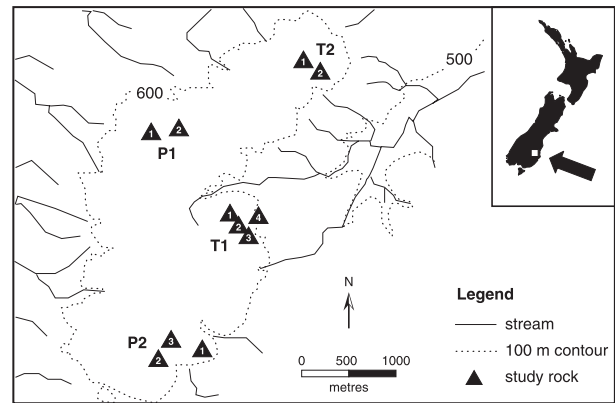
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and dispersal (Waser & Strobeck 1998), and have rapidly become standard tools in molecular ecology.

Most tests of the ability of assignment tests to identify the source of individuals have concentrated on situations where recent dispersal is unlikely (e.g. Manel *et al.* 2002). Typically in these situations, populations are distant and well differentiated genetically, and assignment has proved to be a useful tool for assigning provenance to trophies or smuggled animals, tracking the translocation history of endangered species, and as a general indicator of gene flow between populations (e.g. Paetkau *et al.* 1995; Nielsen *et al.* 1997; Maudet *et al.* 2002). Although these studies have shown that assignment can be highly accurate, they tell us little about the effectiveness of assignment in detecting ongoing dispersal in natural populations.

Dispersal is an important process for species inhabiting patchy environments because it influences both within- and between-patch population dynamics (Turchin 1998). Consequently, measuring dispersal is a major focus of research in conservation biology, pest management and evolutionary biology (e.g. Clobert *et al.* 2001). Because dispersal is notoriously difficult and time consuming to measure directly (Koenig *et al.* 1996), it has been suggested that in some situations assignment could replace field measures of dispersal such as mark-and-recapture (Waser & Strobeck 1998; Cornuet *et al.* 1999). However, to replace field measures, assignment methods first require verification. Thus far, attempts to verify the usefulness of assignment in cases where dispersal is common have been confined to simulation studies (e.g. Cornuet *et al.* 1999), without comparison to empirical field data. Computer simulations in which the probability of correct assignment is measured in relation to dispersal rates have established that as the dispersal rate increases, and genetic differentiation decreases, the ability to distinguish the source of an individual also falls. Furthermore, they indicate that estimates of the level of genetic differentiation between populations (i.e.  $F_{ST}$ ) are useful predictors of the performance of assignment methods that could be taken into account when designing research to measure dispersal (Cornuet *et al.* 1999). However, the assumptions and results of computer models should be validated with empirical data (e.g. Brook *et al.* 2000).

In this study we test the effectiveness of assignment methods for measuring natal dispersal in a situation where populations are well connected by dispersing animals. The species investigated, *Oligosoma grande* (the grand skink), is a rare lizard endemic to subalpine tussock grasslands in southern New Zealand. The skink lives in small groups of about 20 individuals that are restricted to free-standing rock outcrops, and separated from other groups by 50–150 m of inhospitable vegetation. *Oligosoma grande* persists in this patchy environment by the formation of a metapopulation, with regular dispersal between rock outcrops (Whitaker 1996). The species is well suited to investigating



**Fig. 1** Macraes Flat, southern New Zealand, showing the location of the four replicate sites and their study rocks at 'Redbank'. Rocks within sites are separated by between 34 and 356 m, and sites are separated by between 840 and 2460 m.

the accuracy of assignment tests because in addition to having clearly demarcated and easily censused populations, it has been the focus of a detailed long-term (> 13 years) mark-and-recapture study (Whitaker 1996; M. Tocher unpublished results).

Here we compare records of skink natal dispersal generated by mark-and-recapture studies with estimates from several commonly applied assignment tests based on microsatellite DNA data. Specifically, we ask: (i) how accurate is assignment in detecting the natal origin of individuals when compared to mark-recapture data, (ii) how do the accuracies of different assignment tests vary, and (iii), how well does  $F_{ST}$  predict the accuracy of assignment?

## Materials and methods

### Field methods

Our study site was 'Redbank', near Macraes Flat, southern New Zealand (45°25' S, 170°24' E; Fig. 1). Part of the area is designated a reserve for the conservation of lizards, and consists of a mosaic of native subalpine tussock grasslands and agricultural pasture, punctuated by distinctive house-sized schist rock outcrops. Grand skinks are large (up to 111 mm snout-vent length) territorial lizards that inhabit these highly creviced outcrops, and rarely venture into the surrounding matrix vegetation (Whitaker 1996).

We sampled four replicate sites, each of which consisted of a cluster of neighbouring rock outcrops (Table 1). Sites were chosen because they had large resident skink populations and were relatively isolated from other inhabited rocks. Two sites, P1 and T1, were studied during annual field seasons between 1995 and 2001. Each field season was divided into three repeat capture periods, which enabled near complete capture of resident skinks on each rock outcrop. Also, intensive surveys were conducted at two

**Table 1** Summary statistics for 13 microsatellite loci from 11 grand skink populations at Redbank, southern New Zealand

Site	Rock	Site $F_{ST}$	$N$	$N_{\text{natal}}$	$A$	$H_O$	$H_E$
P1	1	0.109 ± 0.021	25.0 ± 0.0	14	7.8 ± 0.5	0.717 ± 0.159	0.774 ± 0.038
	2		37.4 ± 0.3	26	9.0 ± 0.8	0.803 ± 0.108	0.776 ± 0.020
T1	1	0.041 ± 0.006	22.7 ± 0.4	14	8.2 ± 0.7	0.800 ± 0.180	0.793 ± 0.045
	2		21.1 ± 0.3	15	8.1 ± 0.8	0.816 ± 0.082	0.794 ± 0.022
	3		22.0 ± 0.0	15	8.4 ± 0.6	0.860 ± 0.178	0.800 ± 0.040
	4		15.9 ± 0.1	9	8.2 ± 0.7	0.820 ± 0.193	0.800 ± 0.042
P2	1	0.073 ± 0.011	28.6 ± 0.2	13	7.9 ± 0.5	0.834 ± 0.122	0.780 ± 0.026
	2		26.4 ± 0.1	3	6.8 ± 0.4	0.766 ± 0.214	0.728 ± 0.051
	3		18.3 ± 0.2	5	6.2 ± 0.0	0.791 ± 0.188	0.741 ± 0.045
T2	1	0.053 ± 0.006	22.0 ± 0.7	5	7.5 ± 0.5	0.811 ± 0.176	0.768 ± 0.045
	2		15.0 ± 0.0	12	6.8 ± 0.4	0.744 ± 0.207	0.760 ± 0.050

Site  $F_{ST}$  = site-wide  $F_{ST}$ ,  $N$  = average number of skinks genotyped at each locus,  $N_{\text{natal}}$  = number of skinks with known natal rocks,  $A$  = average number of alleles at each locus,  $H_O$  = average observed heterozygosity at each locus,  $H_E$  = average gene diversity at each locus (Nei 1987). All estimates are ± standard error.

additional sites, P2 and T2, during the 2000/2001 summer field season and skinks were captured sporadically at these sites between 1995 and 2001. In most cases there were no inhabited rocks between the sampled rock outcrops, but at site P2 a small number of skinks ( $\leq 5$ ) were present on rocks between the sampled rock outcrops.

Skinks were captured with nylon fishing line nooses, marked with a unique and permanent toe-code and released at their point of capture. During the 2000/2001 field season tissue samples were collected for genetic analyses from all captured skinks. Samples consisted of toe-clips or tail-tips ( $< 2$  mg tissue), and were placed immediately into liquid nitrogen. A total of 261 skinks were genotyped for 13 loci following the methods described in Berry *et al.* (2003). This sample contained virtually all skinks inhabiting each rock outcrop based on mark–recapture estimates of population sizes (O. Berry, unpublished data). The average number of alleles, the observed heterozygosity, and the average gene diversity per rock outcrop were calculated using  $F_{STAT}$  2.9.3 (Goudet 1995).

#### Identifying natal dispersers with mark–recapture data

The natal rock was identified for 131 genotyped skinks from their mark–recapture history. These skinks were first captured in autumn as newborns or in the following spring as young of the year. They could be distinguished because grand skinks have four easily recognizable age/size classes based on their snout–vent length:  $\leq 1$  years old,  $\leq 2$  years old,  $\leq 3$  years old, and adults (Whitaker 1996). We assumed that skinks  $\leq 1$  year old were captured on their natal rock. This assumption is based on the movement patterns of  $\leq 1$ -year-old skinks established from a more extensive mark–recapture dataset including additional sites, where only 3.3% of skinks first caught as newborns in autumn dispersed to another rock during their first year ( $n = 120$ ; M. Tocher,

unpublished data). This natal information was used to categorize skinks as either natal dispersers or natal stayers.

#### Identifying natal dispersers with genotypic data

Genotypic data from all skinks were used to calculate the allele frequencies on each rock outcrop, and two methods were used to assign individuals probabilistically to candidate rock outcrops based on their multilocus genotypes: the partially Bayesian method (Rannala & Mountain 1997), and the fully Bayesian clustering method (Pritchard *et al.* 2000). The partially Bayesian method has been shown to be the most accurate of the frequentist assignment approaches (Cornuet *et al.* 1999), while empirical studies suggest that the fully Bayesian method has equal or higher accuracy (e.g. Eldridge *et al.* 2001). Both of these methods assume Hardy–Weinberg equilibrium and also linkage equilibrium between loci within each population. Separate assignment tests were performed for each site, and also conducted between all pairs of rock outcrops (both between and within sites). This permitted us to examine the performance of assignment tests at both small and large spatial scales, where natal dispersal is common and rare, respectively. The accuracy of assignment was calculated as the percentage of skinks with known natal rocks that were correctly assigned to their natal rock. The  $\phi_2$  correlation coefficient was used as a measure of association (Zar 1996) to examine whether the same individual skinks were consistently assigned (using the most likely criteria) correctly or incorrectly to their natal rock by each assignment method.  $\phi_2$  varies between 0, if there is no association between assignments by each method, and 1 or  $-1$ , if there is complete agreement or disagreement of assignments, respectively. The significance of the association can be assessed by considering the significance of the contingency table with the  $\chi^2$  statistic (Zar 1996).

### Assignment calculations

Partially Bayesian assignments were calculated with GENECLASS (Cornuet *et al.* 1999) using the 'leave one out' option, which sequentially removes the individual under consideration when calculating the allele frequencies for each rock outcrop. Fully Bayesian assignments were calculated using STRUCTURE 1.0 (Pritchard *et al.* 2000). STRUCTURE permits inclusion of a range of prior information as parameters in the model. An estimate was included of the probability that each individual was a migrant to the rock upon which it was captured ( $v$ ). Our field data for skinks with known natal rocks indicated that between approximately 5 and 20% of skinks captured on a rock outcrop were born on another rock at the same site. For STRUCTURE analyses within each site (small spatial scales) uncertainty in these estimates was accounted for by specifying two probabilities that each individual was a migrant ( $v = 0.05$  and  $v = 0.20$ ) in replicate runs of the analyses. No dispersal between sites was recorded in this study, but dispersal of up to 403 m has been recorded previously (Whitaker 1996), and it is likely that occasional longer dispersal events occur. In light of this, and because setting  $v$  greater than zero is recommended by Pritchard *et al.* (2000), it was set to  $v = 0.01$  for all between site (large spatial scale) runs of STRUCTURE. Because previous analyses showed that populations inhabiting each rock outcrop were significantly genetically differentiated from one another (O. Berry, unpublished data), and long-term mark-recapture data show that most skinks remain on a single rock for their lifetime (M. Tocher, unpublished data),  $K$  (the number of populations) was specified according to the number of rock outcrops present at a site, i.e. for analyses involving pairs of populations  $K = 2$ , and for assignments at site T1 and P2, which have four and three rocks, respectively,  $K = 4$  or 3. STRUCTURE was also used to verify that the number of rock outcrops at each site reflected the actual number of genetic groups present by identifying the number of populations with the highest posterior probability. However, in all cases this approach suggested more groups than the number of rocks present. This result was most probably because of the presence of multiple family members on each rock outcrop (O. Berry, unpublished data), which is predicted to over-estimate  $K$  (Pritchard & Wen 2003), although it may be the result of small numbers of migrants from rock outcrops outside the study sites. It was specified that the model should use the rock outcrop where skinks were caught as prior information (USEPOPINFO = 1), and that the allele frequencies on different candidate rock outcrops were correlated (FREQSCORR = 1). The remainder of the input parameters were left at default values. When running STRUCTURE a burnin of 50 000 iterations of the Markov chain followed by a run for 50 000 iterations was used because this produced consistent results in replicate runs.

### Stringency of assignments

Both the fully and partially Bayesian methods of assignment provide a level of confidence in the assignment of each individual. This permits a *priori* specification of the level of confidence, or stringency, required to accept an individual as a natal disperser or stayer. The chosen level of stringency will depend on the application of the data. For example, a very high level of confidence in an assignment may be required if the information is to be used in a court of law (e.g.  $P \leq 0.001$ ; Primmer *et al.* 2000), but a lower level of confidence may suffice in studies of wildlife dispersal (e.g. Galbusera *et al.* 2000; Pritchard *et al.* 2000). The accuracy of assignments was calculated under a range of stringencies for both methods of assignment as follows.

For the partially Bayesian analysis, skinks were assigned to candidate rocks at: (i) the most likely rock, (ii) rocks with  $\geq 80\%$  confidence of exclusion ( $P \leq 0.20$ ), and (iii) rocks with  $\geq 95\%$  confidence of exclusion ( $P \leq 0.05$ ). The exclusion method was introduced by Cornuet *et al.* (1999), and involves simulating a large number of multilocus genotypes from a rock's observed allele frequencies and comparing the probability that a focal skink's genotype originated on a candidate rock to a distribution of probabilities derived from the simulated genotypes. These calculations were performed with GENECLASS using 10 000 simulated individuals, and an assignment was considered correct when only the known natal rock had a probability  $\geq 80\%$  or  $\geq 95\%$  of being the source of a skink. This approach does not assume that the true candidate population has been sampled because it does not compare populations but considers each separately. This may be advantageous in situations where sampling all of the candidate populations is not possible (Cornuet *et al.* 1999).

For the fully Bayesian clustering analysis, skinks were assigned using STRUCTURE 1.0 to rocks at three levels of stringency: (i) the rock with the highest posterior probability of belonging, (ii) rocks with a  $\geq 95\%$  posterior probability of belonging ( $T \geq 0.95$ ), and (iii) rocks with a  $\geq 99\%$  posterior probability of belonging ( $T \geq 0.99$ ). In contrast to the partially Bayesian method, the fully Bayesian method assumes that the true candidate population included in the analysis, and the posterior probabilities that an individual originated from each of the candidate populations sum to one.

### The relationship between genetic differentiation and the accuracy of assignment

Separate assignment tests were run for all pairs of rock outcrops, and the percentage of skinks assigned correctly to their natal rock was calculated under each level of stringency. These data were plotted against  $F_{ST}$  values between pairs of rocks derived by the method of Weir &

Cockerham (1984) using FSTAT 2.9.3 (Goudet 1995). In some cases when pairwise assignment tests were conducted a small number of skinks being considered were known to have migrated from noncandidate rocks within the same site as the candidate rocks (usually < 5%). Because these individuals could not be correctly assigned to either of the candidate rocks they were not included when the accuracy of assignment was calculated.

#### *The effect of the number of loci on the accuracy of assignment*

The effect of the number of loci used on the accuracy of assignments was tested. These analyses were confined to sites P1 and T1 because a large proportion of skinks had known natal rocks at these sites. In addition, these sites differ in their rates of natal dispersal, and have moderate and low levels of genetic differentiation, respectively (P1  $F_{ST} = 0.109$ ; T1  $F_{ST} = 0.041$ ). Five replicate datasets of 1–12 loci from the existing dataset were randomly subsampled without replacement and the percentage of skinks correctly assigned to their natal rock for each subsample was calculated.

## Results

#### *Frequency of dispersal detected by mark-and-recapture*

No between-site dispersal events were recorded by mark-and-recapture during this study, but dispersal was recorded between rock outcrops at all of the sites. The majority of skinks remained on their natal rock but the percentage of skinks that were natal dispersers ranged between 5 and 18.9% at different sites. The mean dispersal distance recorded for dispersing skinks with known natal rocks was 118.4 m ( $\pm 20.6$  SE,  $n = 13$  dispersers). The longest dispersal distance recorded was 235 m at site P1.

#### *Genotypic data*

Between 15 and 38 skinks were genotyped per rock outcrop (Table 1). The populations on each outcrop were highly polymorphic for the microsatellite loci examined, with the average number of alleles per locus on each rock outcrop ranging between 6.2 and 9.0. The average gene diversity ranged between 0.73 and 0.80 (Table 1). Global and by-rock tests showed that the loci were in Hardy–Weinberg equilibrium and in linkage equilibrium.

#### *The accuracy of assignment tests to detect natal dispersal and the effect of high stringency*

Both the partially and fully Bayesian assignment tests without high stringency correctly identified most natal

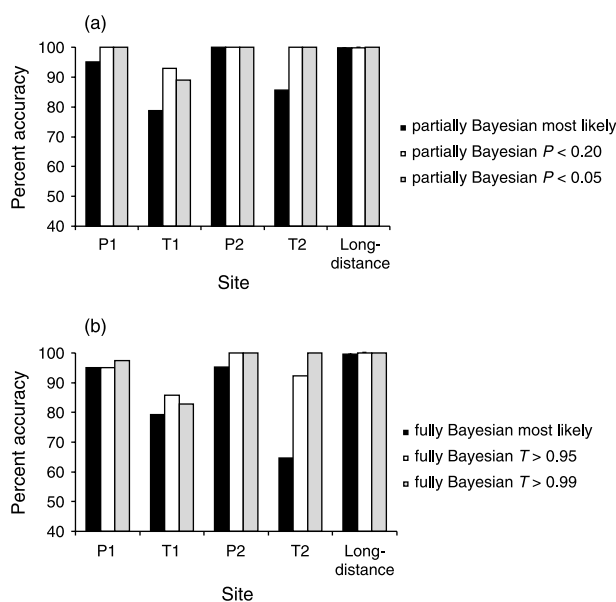


Fig. 2 Percentage of individual skinks correctly assigned to their natal rock at each site, and correctly assigned to their natal rock in pairwise tests between study rocks from different sites (long-distance, average  $\pm$  SE). (a) partially Bayesian method (b) fully Bayesian method.

dispersers and stayers at all of the sites (Fig. 2a,b). The least accurate result was the fully Bayesian method at site T2, which assigned 64.70% of skinks correctly to their natal rock. Both methods assigned more than 95% of skinks correctly to their natal rock at sites P1 and P2 and also for the between-site analysis.

Assignments that met the stringency criteria were more accurate than assignments without high stringency at all sites (Fig. 2a,b). The partially Bayesian method achieved 100% accuracy for both the 80% ( $P \leq 0.20$ ) and 95% ( $P \leq 0.05$ ) exclusion at all sites except T1, where they achieved 70% and 89% accuracy, respectively. Both stringencies of the fully Bayesian method achieved over 92% accuracy at all sites and for the between-site analysis, except at site T1 where they achieved 85% and 82% accuracy, respectively.

Although assignments with higher stringency were more accurate, fewer skinks could be assigned because more had undefined origins (Fig. 3). This was most pronounced for the partially Bayesian exclusion methods. At all sites, and also for the between-site analysis, fewer than 40% of skinks were assigned to one rock outcrop with  $P \leq 0.2$  and fewer than 30% were assigned with  $P \leq 0.05$ . For the fully Bayesian method, the effect of a stringency of  $T \geq 0.99$  and 0.95 was less marked. At all of the sites except T1 more than 90% of skinks were assigned with a posterior probability  $\geq 0.95$ , and at all of the sites more than 78% could be assigned with  $\geq 0.99$  posterior probability. At the larger, between-site scale, on average

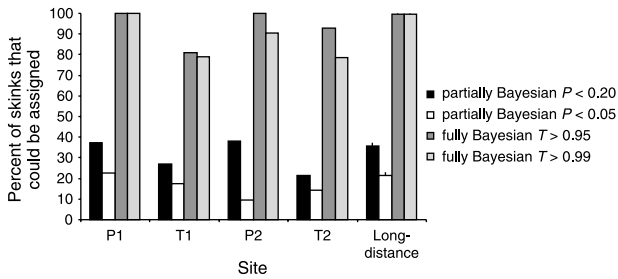


Fig. 3 Percentage of skinks that were assigned to a rock outcrop and met stringency criteria at each site and in pairwise tests between study rocks from different sites (long-distance, average  $\pm$  SE).

more than 99% of skinks were assigned with a posterior probability  $\geq 0.99$ .

Individual skinks at sites P1 and T1 were usually assigned in the same manner (correctly or incorrectly to their natal rock) by both assignment methods using the most likely criteria (site P1  $\phi_2$  correlation = 1.0,  $\chi^2$  (with Yates correction) = 21.72, d.f. = 1,  $P < 0.001$ ,  $n = 40$ ; site T1  $\phi_2$  correlation = 0.46,  $\chi^2 = 8.49$ , d.f. = 1,  $P < 0.004$ ,  $n = 53$ ). Sample sizes were too small for valid tests to be performed for the other sites (P2 and T2). Under the most likely

stringency, neither method of assignment was consistently more accurate at all four sites (compare Fig. 2a,b), and there was no significant difference in the percentage assigned correctly by each method in the between-site analyses (paired  $t$ -test,  $t = 1.54$ , d.f. = 54,  $P = 0.13$ ,  $n = 55$ ).

#### The effect of the number of loci on the accuracy of assignment

The accuracy of partially Bayesian assignments usually improved when more loci were included but the rate of improvement differed for sites T1 and P1 (Fig. 4a,b). The improvement was gradual at site T1, which has a low level of genetic subdivision ( $F_{ST} = 0.041$ ), and improved from *c.* 50% with one locus to 78.8% with 13 loci. In contrast, assignment accuracy improved rapidly at the more genetically subdivided site P1, starting between 70 and 90% and reaching an asymptote at 95% with close to four loci. In addition, while the combination of loci used caused the accuracy to vary by often more than 10% at site T1, the level of accuracy varied little between locus combinations at site P1. In contrast to the partially Bayesian analysis, the accuracy of fully Bayesian analysis showed no improvement when more loci were included (Fig. 4c,d) but using

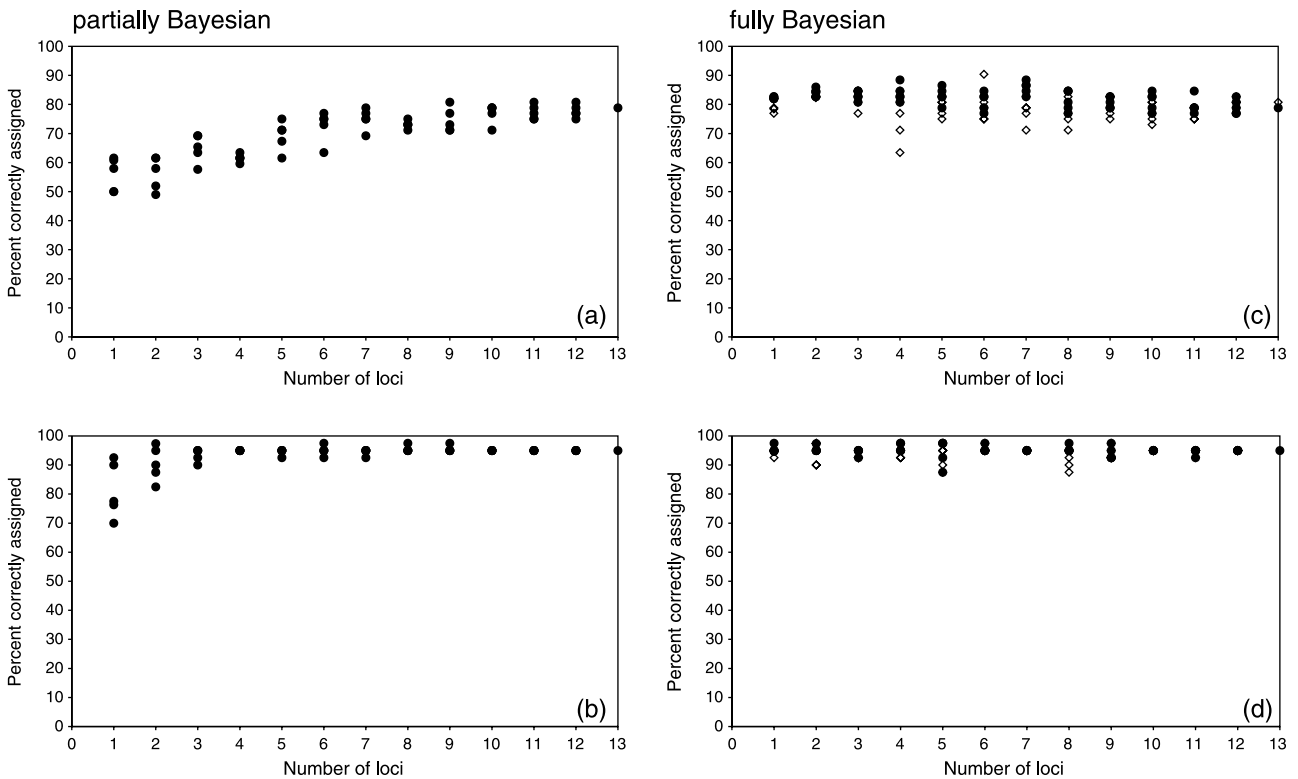
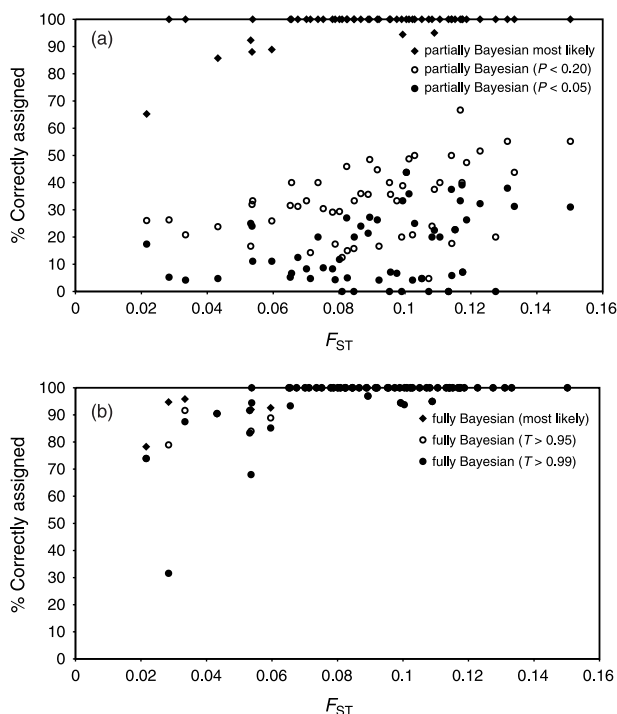


Fig. 4 The relationship between the number of loci used for assignment tests and the percentage of assignments that were correct at: (a) site T1, partially Bayesian method; (b) site P1, partially Bayesian method; (c) site T1, fully Bayesian method; (d) site P1, fully Bayesian method. For the fully Bayesian analyses  $\diamond$  represent tests where a prior migration probability ( $v$ ) of 0.20 was specified and  $\blacklozenge$  represent a prior migration probability of 0.05.

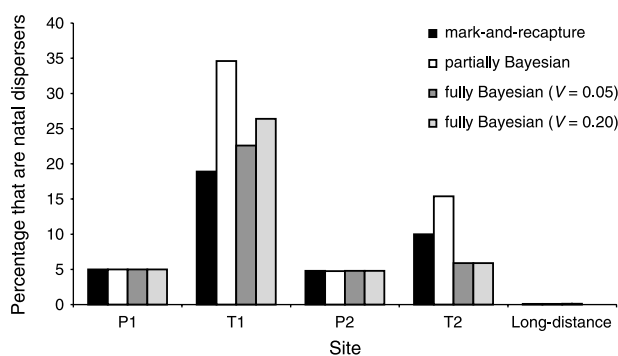


**Fig. 5** The relationship between genetic differentiation and the accuracy of assignments in pairwise tests between all rock outcrops. The effect of different stringencies on the percentage of correct assignments is also shown; (a) partially Bayesian method, (b) fully Bayesian method. In (b), intrasite tests (predominantly  $F_{ST} < 0.1$ ) were run with two prior migration probabilities. For simplicity only the results from the prior migration probability of 0.20 are shown. Assignments were usually more accurate for the  $v = 0.05$ .

a more conservative prior probability of being a natal disperser ( $v = 0.05$ ) usually produced slightly greater accuracy at both sites for all numbers of loci and loci combinations.

#### *The relationship between genetic differentiation and the accuracy of assignment*

The percentage of skinks assigned correctly increased as populations were more genetically differentiated under both the partially and fully Bayesian assignment approaches (Fig. 5a,b). Both methods with the most likely stringency reached close to their maximum accuracy of 100% at an  $F_{ST}$  between 0.06 and 0.08. Applying higher stringencies to the partially Bayesian analysis greatly reduced the proportion of skinks that could be assigned to any candidate rock outcrop, thus reducing the efficiency of this method to identify dispersers (cf. Figs 2a,b and 3). Applying higher stringencies to the fully Bayesian method reduced the efficiency of assignment relatively little because most skinks were assigned to one of the candidate rocks, and above  $F_{ST}$  of 0.07 only four of 44 comparisons were less



**Fig. 6** The percentage of skinks that were identified as natal dispersers by mark-and-recapture or that were assigned to a rock outcrop other than where they were captured at each site and between all pairs of rock outcrops between sites (long-distance, average  $\pm$  SE). For the fully Bayesian analyses the results for two prior migration probabilities ( $v$ ) are shown.

than 100% accurate with  $T = 0.99$ , and all of these were more than 90% accurate.

#### *Estimating the number of dispersers from misassignments*

If skinks assigned to rocks other than those upon which they were captured were considered to be dispersers (cf. Cornuet et al. 1999; page 1999), both assignment methods estimated numbers of dispersers similar to those estimated by the mark-and-recapture method within each site (Fig. 6). The greatest exception was at site T1, where the partially Bayesian, and to a lesser extent the fully Bayesian method, overestimated the proportion of individuals that were dispersers. The prior estimate of dispersal probability ( $v = 0.05$  or 0.20) generally had little effect on the estimate of the number of dispersers for the fully Bayesian method. While there were no intersite dispersers recorded in the mark-recapture data, a small number of dispersers were identified by both the partially and fully Bayesian methods, which both on average estimated 0.13 ( $\pm 0.13$  SE) per cent of individuals dispersing between pairs of sites.

#### **Discussion**

Genetic studies are often viewed as a quick means to measure dispersal (Steinberg & Jordan 1998). For example, the fieldwork necessary for the molecular component of our study was completed in less than 3 months, whereas the mark-and-recapture data were generated over more than 7 years. If these methods produce similar results, then assignment tests would represent a major saving of effort and expense and reduce the invasiveness of field studies. Our combined microsatellite DNA data and long-term mark-and-recapture records provide strong evidence that assignment tests can provide dispersal data of high

accuracy. They show that in most cases, assignment tests were reliable indicators of the origin of individuals, even when populations regularly exchanged migrants. However, that accuracy depended on the stringency applied, the level of genetic differentiation between populations, and the number of loci used.

#### *Using assignment tests for qualitative and quantitative measures of dispersal*

Assignment tests provided a qualitative idea of the spatial scale of skink dispersal consistent with expectations from mark-and-recapture studies. Very few skinks (average 0.13%) were assigned to a rock outside their site (distances 840–2460 m), but more were assigned to a rock outcrop within their site (distances 34–356 m), indicating that dispersal of distances greater than 800 m is rare. This matches the pattern found in the mark-and-recapture data, and had we known little about rates of dispersal in grand skinks, this information would have been valuable in defining the area necessary to establish a reserve for these endangered lizards (Whitaker & Houston 2002). However, the consequences of dispersal for the dynamics of populations can best be understood with quantitative estimates of dispersal (Turchin 1998). Therefore, one of our most important results was that assignment tests without high stringency provided estimates of the number of natal dispersers that were comparable to those obtained by mark-and-recapture. Like previous studies on rock wallabies and ibex (Eldridge *et al.* 2001; Maudet *et al.* 2002), this was true not only at the large spatial scale where natal dispersal must be very rare or absent, but importantly it was also true at each site where between 4.8 and 18.9% of skinks disperse from their natal rocks. In addition, at the level of individual skinks, the accuracy of assignment tests was high, with most individuals being assigned correctly to their natal rock. Although similarly high accuracy has been demonstrated previously, it has usually been between more differentiated populations that exchange few migrants (Eldridge *et al.* 2001; Manel *et al.* 2002).

#### *The effect of population differentiation on assignment accuracy*

Our data demonstrate a positive relationship between levels of  $F_{ST}$  and the ability to assign individuals correctly to their natal population, similar to predictions made from simulations using both infinite allele and stepwise mutation models (Cornuet *et al.* 1999). Hence assignment tests are robust to key assumptions about the model of mutation for the markers, and have general application. We also showed that assignments were usually 100% accurate for low to moderate levels of genetic differentiation ( $F_{ST} \sim 0.07$ ), and still moderately accurate (~78%) for populations with

lower levels of genetic subdivision (e.g. site T1,  $F_{ST} = 0.04$ ) that correspond to reasonably high levels of natal dispersal (18.9%). These results suggest that assignment methods have wide applicability in population studies.

Our assignments were more accurate than those from previous empirical studies. In the study by Maudet *et al.* (2002) for reintroduced ibex populations and where  $F_{ST}$  between populations were usually greater than those under study here ( $F_{ST} = 0.167$ ), assignments were 74.4% accurate for the fully Bayesian method with  $T \geq 0.99$ . Similarly, across a range of datasets where  $F_{ST}$  values were usually greater than those reported here (average = 0.270), the mean percentage of individuals correctly assigned by the fully Bayesian method at  $T \geq 0.99$  was also lower (c. 75%; Manel *et al.* 2002). Notably, the heterozygosities reported for loci in those studies were lower than our values (averaging 0.40 and 0.47, respectively, vs. 0.77), supporting the notion that loci with higher levels of variation (measured by number of alleles or gene diversity) are better at discriminating the source of individuals (Estoup *et al.* 1998; Bjørnstad & Røed 2002). Thus, where possible, highly variable loci should be used in assignment analyses.

#### *The number of loci required for assignment*

Simulation studies have shown that additional loci improve the ability to assign individuals correctly for a given level of differentiation between populations (Cornuet *et al.* 1999). Conceivably, assignment could accurately measure higher rates of dispersal if sufficient loci were used. Yet our results show that the rate of improvement with the addition of loci is gradual when genetic differentiation is low, and suggest that many loci would be necessary for species where dispersal is high, possibly incurring high financial costs and so diminishing any potential advantage over field-based measures of dispersal. Currently, most microsatellite DNA-based molecular surveys use fewer than the 13 loci used here; the average number used to study animal population genetic structure was 8.00 ( $\pm 0.48$  SE,  $n = 35$ ) in *Molecular Ecology* publications in 2001. Because there are many organisms for which molecular markers have not been developed, the additional cost and time required for marker development may limit the usefulness of genetic measures of dispersal. However, as more efficient protocols for microsatellite development are used and coamplification of multiple loci becomes more common, the cost is likely to decrease (Henegariu *et al.* 1997; Zane *et al.* 2002).

#### *Different methods of assignment and the effect of high stringency*

Testament to the usefulness of assignment tests is the rapid proliferation of methodological variants that have

been developed (e.g. Bowcock *et al.* 1994; Rannala & Mountain 1997; Pritchard *et al.* 2000). We found that the fully Bayesian approach of Pritchard *et al.* (2000) and the partially Bayesian approach of Rannala & Mountain (1997) usually produced equally accurate assignments when the most likely criterion was applied. Both were also highly accurate at higher stringencies, but the methods differed greatly in the proportion of individuals that they could assign with these higher stringencies. In part this is explained by an important difference in the assumptions behind the derivation of confidence in these assignment methods; the fully Bayesian method assumes that the true candidate population has been sampled, whereas the partially Bayesian method does not. Because we had complete knowledge of the provenance of all individuals, we could be confident that all the appropriate candidate rock outcrops were sampled, and we removed seven skinks from our analysis that were known to have dispersed from outside the study rocks. This fulfils a key assumption of the fully Bayesian method. When the skinks that originated from outside the study rocks were not excluded from the analysis, the partially Bayesian correctly excluded them all from the rock on which they were captured at  $P \leq 0.05$ , while the fully Bayesian method incorrectly assigned six skinks to the rock on which they were captured with high probability ( $T \geq 0.99$ ). Comparison of these results is problematic because the assumptions of the two assignment methods differ. However, even when the known outside-site skinks were included, the partially Bayesian method remained a much more conservative approach to assignment with high confidence because only a small proportion of individuals could be assigned. Hence, when assignments require high confidence the method of assignment can have a large bearing on the number of individuals that can be assigned. Furthermore, the likelihood that all appropriate populations have been sampled should be an important consideration when deciding on the method of assignment to be used.

High stringency analyses are important in situations where the cost of an incorrect assignment are high, such as in forensic cases where legal action is undertaken (Primmer *et al.* 2000). Although our results show that assignments with high stringency were more accurate than assignments without stringency, this advantage can be subtle, and must be traded against the sometimes large reduction in the number of individuals that can be assigned. In the case of the partially Bayesian methods, fewer than half of the skinks could be assigned to a natal rock. Manel *et al.* (2002) reported similarly high numbers of individuals of undefined origin, and simulations also show many un-assigned individuals (Cornuet *et al.* 1999).

#### *Comparing assignment data with mark-and-recapture data*

Although assignment tests provided quantitatively similar estimates of dispersal rates to those provided by the field data, examination of the assignment of individuals reveals that sometimes up to one-third of assignments were incorrect at sites with less genetic subdivision (cf. Fig. 2a,b). Based on our field data, both natal dispersers and natal stayers were sometimes incorrectly assigned. These errors cancelled each other when the total number of 'dispersers' was tallied. In addition, quantifying the error rate is complicated by the possibility that errors exist in the mark-recapture data. Our preliminary data showed that a small proportion (c. 3%) of our field-based natal classifications would be incorrect because some newborn skinks disperse in their first year. Furthermore, natural toe loss occurs occasionally in grand skinks and some individuals may be misidentified. Indeed, some individuals not identified as dispersers by the field data were consistently labelled dispersers by all assignment methods, and for many combinations of loci, indicating that their genotype was atypical for the rocks upon which they were resident. These data strongly suggest that some dispersal events were not detected by mark-recapture. Although we believe there were very few errors in the mark-and-recapture data, they inevitably do occur, and the assignment data provides a useful check of dispersal records. For example, one skink identified by the mark-and-recapture data at site P1 as a disperser was never identified as a disperser by assignment tests. As this site had a moderately high level of genetic subdivision ( $F_{ST} = 0.11$ ), and assignments were highly accurate, this leads us to suspect that an error was made in the field data. In addition, the offspring and descendents of dispersers will sometimes be incorrectly assigned because they will have genotypes typical of more than one population. This should inflate the number of misassigned skinks over the true dispersal rate, particularly when dispersal rates are high and matings with migrants are common (cf. Fig. 6). If populations can be well sampled, one approach to identifying such individuals would be to use parentage or kinship analysis (e.g. Marshall *et al.* 1998; Goodnight & Queller 1999), in combination with assignment tests. We are investigating this approach as part of a study into the mating system of this species.

#### *Conclusions*

Estimating rates of dispersal is critical to understanding the dynamics of patchy populations, yet dispersal is difficult and time consuming to measure (Koenig *et al.* 1996). We have shown that in a short time period, assignment tests can identify the natal population of most individuals,

and provide estimates of the proportion of migrants that are similar to results from a long-term mark-and-recapture study. Because assignment tests require significantly less fieldwork than traditional mark-and-recapture approaches to measuring dispersal (here < 3 months vs. > 7 years), they will provide useful qualitative and sometimes quantitative estimates of dispersal in many applied and theoretical situations. However, several caveats apply. (i) Assignment tests are more accurate when populations are more genetically differentiated, although they can still be highly accurate at low to moderate levels of genetic differentiation when dispersal is common. (ii) Assignment accuracy can be improved by increasing the number of loci used, but if populations have a low level of genetic differentiation the number of loci required may be prohibitive. (iii) Assignment accuracy can also be improved by applying high stringency, but the method used (fully or partially Bayesian) can have a large bearing on the number of individuals that can be assigned.

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