Population genetic structure of tailed frogs (*Ascaphus truei*) in clearcut and old-growth stream habitats in south coastal British Columbia

T.R. Wahbe, C. Ritland, F.L. Bunnell, and K. Ritland

Abstract: *Ascaphus truei* Stejneger, 1899 relies on cool, fast-flowing, forested mountain streams, which receive little protection from logging activities. During recovery of post-logging habitats, local extirpation of *Ascaphus* is a concern because their recolonization may be slow. In British Columbia, Oregon, and California, coastal *Ascaphus* populations are designated as "at risk". To infer fragmentation impacts from patterns of genetic structure, we used RAPD molecular markers and compared population genetic structures of *Ascaphus* larvae in an old growth with an adjacent clearcut stream located in coastal British Columbia. In the clearcut, larvae were less genetically diverse than in the old growth and exhibited no relationship between physical distance and genetic relatedness, whereas in the old growth, genetic similarity decreased with physical distance. Patterns of gene flow between the clearcut and old-growth streams were significantly different. The clearcut population went through a bottleneck/founding event, but also exhibited greater dispersal. The frogs were perhaps searching for new habitat (which would lower isolation by distance) and suffering mortality (which would decrease diversity). Most problems in conservation will require genetic and ecological data, and future research should aim to incorporate methodologies from both fields.

Résumé : *Ascaphus truei* Stejneger, 1899 a besoin de cours d'eau forestiers de montagne, à eau fraîche et à courant rapide et ces habitats sont mal protégés des activités de coupe forestière. Durant la récupération des habitats après des opérations de coupe, la disparition locale des *Ascaphus* est un objet de préoccupation, car leur recolonisation peut être lente. Les populations côtières d'*Ascaphus* de la Colombie-Britannique, de l'Oregon et de la Californie sont considérées « à risque ». Afin d'obtenir les impacts de la fragmentation à partir des patrons de structure génétique, nous avons utilisé des marqueurs moléculaires RAPD et comparé les structures génétiques moléculaires de larves d'*Ascaphus* dans une forêt ancienne et dans un cours d'eau adjacent soumis à la coupe à blanc dans la région côtière de la Colombie-Britannique. Dans la région coupée, les larves ont une diversité génétique inférieure à celle trouvée dans la forêt ancienne; il n'y a pas non plus de relation entre la distance physique et la parenté génétique, alors que, dans la forêt ancienne, la similarité génétique décroît en fonction de la distance. Les patrons de flux génétique diffèrent significativement dans les cours d'eau de la zone coupée et ceux de la forêt ancienne. La population de la zone coupée a subi un goulot d'étranglement et un événement fondateur, mais elle montre aussi une plus forte dispersion. Les grenouilles cherchent peut-être un nouvel habitat (ce qui réduirait l'isolement en fonction de la distance) ou subissent de la mortalité (ce qui diminuerait la diversité). La plupart des problèmes de conservation nécessiteront l'apport des données génétiques et écologiques et la recherche future devrait chercher à intégrer les méthodologies de ces deux disciplines scientifiques.

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Introduction

Amphibian species often live in habitats that are altered or fragmented by human activities. Some authors (e.g., Welsh 1990) have argued that recolonization of logged sites is critical to sustaining productive amphibian populations. However, recolonization of sites following local extinction may be difficult for amphibians for several reasons. Suitable habitat is patchy, and the physiological constraints of amphibians hamper movements among these habitats. This results in many amphibians moving short distances (Sinsch 1990) and exhibiting high site fidelity (Blaustein et al. 1994).

The genus *Ascaphus* Stejneger, 1899 ranges from British Columbia to western Montana, between the Pacific Coast

T.R. Wahbe[1,2] and F.L. Bunnell. Centre for Applied Conservation Research, Faculty of Forestry, University of British Columbia, 2424 Main Mall, Vancouver, BC V6T 1Z4, Canada.
C. Ritland and K. Ritland. Genetic Data Centre, Faculty of Forestry, University of British Columbia, 2424 Main Mall, Vancouver, BC V6T 1Z4, Canada.

[1]Corresponding author (e-mail: wahbe@interchange.ubc.ca).
[2]Present address: Institute for Aboriginal Health, BC ACADRE, College of Health Disciplines, University of British Columbia, 400-2194 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada.

and the Rocky Mountains, and southward to northern California (Corkran and Thoms 1996). The characteristic *Ascaphus* habitat is cool, fast-flowing, perennial streams in mountainous, coniferous forests, at elevations ranging from near sea level to above 2140 m (Nussbaum et al. 1983; Leonard et al. 1993; Corkran and Thoms 1996). In coastal British Columbia, *Ascaphus truei* Stejneger, 1899 inhabit sites from near sea level to subalpine zones (30–1100 m; Sutherland 2000).

Ascaphus, the only representative of the family Ascaphidae (Nussbaum et al. 1983), is a highly distinct lineage (Ford and Cannatella 1993; Jamieson et al. 1993) that has retained many primitive morphological features (Green et al. 1989; Ford and Cannatella 1993). *Ascaphus* differ from most Pacific Northwest frogs in that fertilization is internal. They have a low desiccation tolerance (Claussen 1973), and one of the lowest and narrowest temperature tolerances among anurans (Brattstrom 1963; de Vlaming and Bury 1970).

Ascaphus may live 15–20 years (Daugherty and Sheldon 1982*b*) and do not reach reproductive maturity until they are 7 or 8 years of age (Daugherty and Sheldon 1982*b*; Brown 1990). They are believed to be sensitive to habitat alteration because of their lengthy larval period of 1–5 years (Wahbe 1996; Wallace and Diller 1998; Bury and Adams 1999), small clutch size of 30–70 eggs (Metter 1967; Leonard et al. 1993), biennial reproduction at high-elevation inland sites (Rocky Mountains (Metter 1967); reproduction may be annual elsewhere (Bury et al. 2001)), low recolonization potential (Daugherty and Sheldon 1982*a*), and specialized characteristics of their habitats (cool, fast-flowing, perennial mountain streams; Bury and Corn 1988; Hawkins et al. 1988). *Ascaphus* are believed to persist best in relatively stable habitats, such as old-growth forests. Available evidence from inland populations indicates that adult *Ascaphus* remain within a narrow range of 0–20 m around streams (Daugherty and Sheldon 1982*a*). Evidence from coastal sites suggests long-distance overland movements (≥100 m) are more likely where forested stands are present (Wahbe et al. 2004). *Ascaphus* movement potential is generally believed to be low (Daugherty and Sheldon 1982*a*; Bury and Corn 1988).

A primary goal of conservation genetics is to estimate the level and distribution of genetic variation within and among populations of rare and endangered taxa (Fritsch and Rieseberg 1996), and to infer processes of population history based upon these patterns of variation. Research on *Ascaphus* has focused on genetic differences among watersheds across portions of the species' range. Having estimated relationships among populations, Neilson et al. (2001) suggested recognition of inland populations of British Columbia, Idaho, Montana, Washington, and Oregon as a distinct species, *Ascaphus montanus* Mittleman and Myers, 1949. However, Ritland et al. (2000) reported that inland populations in British Columbia were no more distinct from coastal populations than predicted from isolation by geographic distance alone, as opposed to taxonomic differentiation. Nonetheless, both studies reported strong genetic differences (i.e., low gene flow) among *Ascaphus* populations, suggesting a complex history of restrictions to geographic refugia and range expansions.

In contrast, little attention has been paid to within-population processes. Wahbe and Bunnell (2001) recorded shorter larval movements in clearcut streams than in old-growth streams. Wahbe et al. (2004) reported stronger stream

affinity, suggesting lower colonization potential, of juvenile frogs in clearcuts. Timber harvesting may reduce habitat patch size and habitat connectivity, thus reducing movement among fragments and increasing the probability of local extinction (e.g., Sjögren 1991; Bunnell et al. 1992; Fahrig and Merriam 1994). Connectivity between habitat patches is believed to be key to metapopulation persistence (Sjögren 1991) because it allows dispersal between populations (Taylor 1990; Hanski and Gilpin 1991). However, while there is general support for this claim, there remains a diversity of opinions on this controversial subject (see Reed 2004). *Ascaphus* populations may exist in a metapopulation structure (Metter and Pauken 1969; Daugherty and Sheldon 1982*a*; Ritland et al. 2000). Because direct estimates of dispersal can be problematic, Vos et al. (2001) recommended using genetic techniques to determine influences of landscape connectivity on animal dispersal.

For conservation genetic studies, randomly amplified polymorphic DNAs (RAPDs) provide relatively robust DNA-level data for estimating levels and patterns of genetic variation (Fritsch and Rieseberg 1996). Compared with other commonly used techniques, the RAPD technique can generate essentially unlimited numbers of loci, provides a more random sample of the genome, requires a relatively small amount of genomic DNA (AFLPs require 4× more tissue), is economical, and requires simple and relatively fast procedures. Because *Ascaphus* populations are designated as "at risk" and are protected from destructive sampling in British Columbia, we were limited by the amount of tissue and the number of larvae we could collect. Also limiting was the low larval density ($0.5–2.7/m^2$) of *Ascaphus* within the study area (Wahbe and Bunnell 2003). The RAPD technique was well suited for our genetic study based on these restricting factors.

We examined the population genetic structure of *Ascaphus* in one clearcut stream and one old-growth stream in a single watershed, by sampling larvae within each stream along a 180 m transect. Our purpose was to assess whether we could infer fragmentation impacts from patterns of genetic structure. We tested two hypotheses: (1) because the number of breeding adults in old-growth forests was twice that in clearcuts (Wahbe et al. 2004), we predicted that *Ascaphus* larvae in the clearcut stream would be less diverse than larvae in the old-growth stream, and (2) because female *Ascaphus* deposited eggs in the upper headwaters of streams and larvae moved downstream (Wahbe and Bunnell 2001), we predicted that larvae would exhibit lower genetic relatedness with increasing physical distance along a 180 m stream transect.

Materials and methods

Sampling

Ascaphus tissue samples were collected from two south-coastal British Columbia streams within the Mamquam River drainage in the Coast Mountains of British Columbia, near the city of Squamish. We selected one stream flowing through a clearcut site (stand age ~10 years) and a second stream flowing through old-growth forest (250+ years). These sites were ~1.6 km apart. Aside from forest age and stream aspect (northwest for clearcut; southeast for old

growth), stream and site attributes differed little between the two sites.

Ten reaches were established within each stream for tissue sampling positioned 20 m apart, starting at 0 m moving downstream to 180 m. We attempted to sample a minimum of 100 *Ascaphus* larvae per stream, but fewer were found owing to low *Ascaphus* densities in the area (Wahbe and Bunnell 2003). In all, we collected 63 individuals from the old-growth forest and 87 individuals from the clearcut. Tissue was collected by clipping two or three 2 mm notches (~5 mg) from the tails of *Ascaphus* larvae. Samples were preserved in 95% ethanol and stored at –70 °C until DNA extraction. We used samples of all 150 individual *Ascaphus* larvae from two streams residing in one watershed for subsequent DNA assays.

All animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care. We held the required certificate from the University of British Columbia Committee on Animal Care (#A00110), and the British Columbia provincial permit (#C088692) necessary for fieldwork on a species designated as "at risk".

Assay for RAPD molecular markers

DNA extraction from clippings of larval *Ascaphus* tailfins followed Sambrook et al. (1989). DNA was quantified and yield was estimated using a spectrophotometer (Pharmacia Biotech, Ultrospec® 3000) and electrophoresed on a 0.8% agarose gel. On average, 10 mg of tail clippings yielded 100 ng of DNA. The RAPD marker assay followed Williams et al. (1990), and all PCR reactions were performed on the same Programmable Thermal Controller (PTC 100, MJ Research, Inc., Hercules, California).

Following PCR, the products were electrophoresed on 2% agarose gel (24.5 cm × 19.7 cm × 2.0 cm) in 1× TBE buffer at 140 V for 3.5 h. Gels were then stained with ethidium bromide and photographed using ultraviolet light (Epi Chemi II Darkroom). Images were saved electronically, and light levels were standardized across all images using Photoshop® (version 6.0) prior to scoring. RAPD bands were manually sized and scored as presence versus absence of banded phenotypes. All assays were performed by T.R. Wahbe at the University of British Columbia (UBC) Genetic Data Centre. To ensure consistency, T.R. Wahbe also scored all gels. We established gel schemes that would most evenly sample individuals from both clearcut and old-growth sites, and from multiple sampling distances. Both negative (lacking DNA) and positive (high-quality DNA) controls were used with each PCR set.

Among 16 primers screened against a subset of individuals, six primers were used for the full analysis (UBC primers 211, 213, 221, 268, 352, and 400). The sequences are viewable at http://www.michaelsmith.ubc.ca/services/NAPS/Primer_Sets/Primers.pdf. Overall, the six primers yielded 97 zones of RAPD band activity.

To score RAPD markers as presence and absence data, we made the following assumptions (following Lynch and Milligan 1994): (*i*) each band amenable to scoring represented an independent diallelic locus, (*ii*) marker alleles from different loci did not overlap, and (*iii*) the interpretation of banding patterns on gels was unambiguous. We visually scored monomorphic and polymorphic RAPD markers from electronic gel images. A blind scoring method was used, in which the scorer was unaware of the identity or origin of the individuals (e.g., old growth, 60 m). Regardless of intensity, if any bands were present in negative control lanes, those loci were omitted from the data set. The presence or absence of bands was scored, but only relatively easy to score bands (i.e., usually bright and discrete) were considered. Images were grouped by primer, and all possible markers for one primer were scored prior to scoring markers generated from a different primer.

Statistical analyses

Allele frequencies in old growth and clearcut were calculated using the program TFPGA (Tools For Population Genetic Analysis; Miller 1997). Allele frequencies were estimated using the Lynch and Milligan (1994) formula, which takes into account the dominance of RAPD markers and the statistical bias introduced by small sample size. Nei's genetic distance (Nei 1978) between stream reaches were calculated with TFPGA. One-way ANOVA was used to test for differences in heterozygosity between habitats. All reported *P* values were obtained using statistics tested against a preset level of significance, $\alpha = 0.05$.

F_{ST} values, which measure the degree of population divergence, were obtained with TFPGA by applying Weir and Cockerham's (1984) methods of calculating Wright's (1931) *F* statistics. Assumptions of Hardy–Weinberg equilibrium were made by TFPGA when calculating F_{ST} values. Standard errors of F_{ST} estimates were calculated using jackknifing where replicates were obtained from sequential elimination of loci. The 95% confidence intervals were determined by the bootstrap method, with individuals within populations resampled.

Because of the relatively recent recolonization, and colonization of northern habitats, *Ascaphus* populations are probably not at equilibrium (Ritland et al. 2000). Therefore, when using dominant markers, Φ_{ST}, an analog to F_{ST} that does not rely on Hardy–Weinberg equilibrium, may be an appropriate alternative. We used the program WINAMOVA (Excoffier 1996) to perform an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to estimate Φ_{ST}. Φ_{ST}, like its analog, F_{ST}, indicates genetic differentiation. AMOVA is essentially a multivariate analysis of variance (MANOVA), but it uses the pairwise distance matrix as input (Smouse et al. 2001). The program AMOVA-PREP (Miller 1998) was used to prepare input files for WINAMOVA.

The band-sharing coefficient is the main index of similarity used to describe DNA fingerprints from two individuals (Bruford and Beaumont 1998). It gives the probability that a band will be shared for a certain molecular base pair size compared with a known ladder. Shared bands are those that have co-migrated (within 0.5 mm) and have no more than two-fold intensity difference (i.e., the difference between a homozygote and a heterozygote; Bruford and Beaumont 1998). The coefficient describes behavior, or how the phenotypic difference changes with distance (behavior in spatial pattern), and calculates the frequency of shared dominant phenotypes. Band-sharing coefficients were calculated using a program written in Fortran by K. Ritland. Standard errors of the estimates were obtained by bootstrapping, where 100 repeated random samples were selected from the data and the model was estimated from each one (Norusis

Table 1. Mean heterozygosity (*H*) and percentage of polymorphic loci in *Ascaphus truei* sampled at each stream-reach station for old growth and clearcut.

	H^a	S_{mean}	Percentage of polymorphic loci[b]	n^c
Old growth				
0 m	0.2396		49.18	9.80
20 m	0.1493		31.15	10.00
40 m	0.1169		24.59	6.80
60 m	0.1818		37.70	10.00
80 m	0.0000		0.00	1.00
100 m	0.0486		9.84	1.97
120 m	0.2620		57.38	5.90
140 m	0.2578		57.38	6.66
160 m	0.1994		47.54	4.41
180 m	0.1908		40.98	4.00
Mean[d]	0.3109	0.0263	72.13	60.54
Clearcut				
0 m	0.0575		13.11	10.00
20 m	0.0673		14.75	9.67
40 m	0.0849		16.39	3.62
60 m	0.1643		36.07	9.64
80 m	0.1781		36.07	9.97
100 m	0.1679		34.43	9.98
120 m	0.2130		44.26	10.00
140 m	0.1288		26.23	5.00
160 m	0.1323		27.87	10.00
180 m	0.1965		42.62	7.30
Mean[d]	0.2275	0.0259	60.66	85.18

[a]Nei's (1978) unbiased heterozygosity.
[b]Percentage of loci that were not fixed for one allele; based on 95% criterion.
[c]Total number of samples used to calculate estimates.
[d]Means were based on estimates obtained for each locus; not based on means for each stream-reach station.

1993). The relationships between genetic relatedness and physical distance were analyzed using Pearson's correlation coefficients (Norusis 1993).

Results

Polymorphic loci and heterozygosity

Out of a possible 97 RAPD loci, we scored 61 with high confidence. No loci were unique to sampling location (old growth or clearcut), which is expected because of the close proximity of the two streams. Of 61 loci, 72.13% (44) were polymorphic in the old growth compared with 60.66% (37) in the clearcut (Table 1). Genetic diversity (expected heterozygosity, *H*) for old growth and clearcut is given in Table 1. In old growth, the estimate at 80 m is 0.0000 because only one *Ascaphus* larva was available for the calculation. Based on mean heterozygosity, larvae in the clearcut were genetically less diverse, or more closely related (0.2275 ± 0.0259), than larvae in old growth (0.3109 ± 0.0263; $F = 4.259$, $P = 0.041$). Mean heterozygosity per stream reach was reduced by more than 25%.

Table 2. Genetic differentiation between old growth and clearcut ("population"), among stream reaches ("subpopulations") pooled within old growth and clearcut, and among stream reaches analyzed separately within old growth and within clearcut, as given by F_{ST} values.

			95% Confidence intervals[b]	
Unit	F_{ST}	$SE_E{}^a$	Lower	Upper
Population	0.0677	0.0169	0.0383	0.1034
Subpopulation	0.3228	0.0211	0.2835	0.3658
Old growth	0.3136	0.0232	0.2689	0.3613
Clearcut	0.2285	0.0185	0.1945	0.2632

[a]Standard errors of the parameter estimate (SE_E) were based on jack-knifing over loci.
[b]Confidence intervals were based on bootstrapping over loci (1000 iterations).

Frequency of dominant alleles

The distribution of RAPD marker allele frequencies showed the frequency of the dominant allele ranged 0.01–1.00 (fixed). Fewer loci were nearly fixed for the dominant allele in the old growth (15/61 = 24.59%) than in the clearcut (22/61 = 36.07%). These loci contribute little information about population structure and genetic differentiation, as none are specific to either site. Furthermore, most loci were not sufficiently variable to be informative.

Population differentiation

Genetic differentiation estimates between "populations" (forest cover types) and among "subpopulations" (stream reaches), based on F_{ST} values, are provided in Table 2. The degree of differentiation was high among stream reaches ($F_{ST} = 0.3228 ± 0.0211$), but low between forest cover types ($F_{ST} = 0.0677 ± 0.0169$). Forest cover types were analyzed more appropriately separately, revealing that the level of differentiation among stream reaches in the clearcut appeared to be lower ($F_{ST} = 0.2285 ± 0.0185$) than among stream reaches in the old growth ($F_{ST} = 0.3136 ± 0.0232$; Table 2). The hierarchical AMOVA confirmed that most of the genetic diversity (97.11%) was found among reaches within each stream. Genetic variation between the two populations (old growth and clearcut) was small (2.89%).

Genetic relatedness along stream transects

Similarity of RAPD bands within and among stream-reach stations are given in Table 3. For both forest cover types, larvae sampled from the farthest upstream reach (0 m) were more genetically similar than larvae sampled from farthest downstream reach (180 m). Frequency of RAPD band sharing was averaged for individuals at increasing distances from one another and summarized in Table 4. These values are for distance categories (e.g., 0 m apart, 20 m apart) and do not represent sampling stations. On average, frequency of band sharing was greater for larvae sampled in the clearcut stream than in the old-growth stream, regardless of their physical distance (Table 4). In the old-growth stream, larvae sampled within one reach station had a high frequency of band similarity, while larvae sampled at stations of increasing dis-

Table 3. Similarity of RAPD bands within and among stream-reach stations in clearcut and old growth, where reach 0 m is the top of the stream sampling area.

Clearcut, stream-reach stations									
0 m	20 m	40 m	60 m	80 m	100 m	120 m	140 m	160 m	180 m
0.921	0.934	0.928	0.917	0.916	0.917	0.912	0.918	0.922	0.914
0.940	0.953	0.944	0.929	0.931	0.936	0.924	0.927	0.934	
0.927	0.946	0.927	0.920	0.922	0.923	0.918	0.919		
0.938	0.948	0.937	0.924	0.925	0.926	0.914			
0.948	0.956	0.948	0.929	0.933	0.933				
0.946	0.954	0.945	0.930	0.927					
0.940	0.947	0.934	0.929						
0.963	0.964	0.957							
0.968	0.978								
0.968									

Old-growth, stream-reach stations									
0 m	20 m	40 m	60 m	80 m	100 m	120 m	140 m	160 m	180 m
0.876	0.892	0.905	0.890	0.885	0.887	0.852	0.835	0.830	0.856
0.836	0.843	0.851	0.850	0.842	0.815	0.823	0.817	0.810	
0.835	0.848	0.859	0.853	0.856	0.858	0.812	0.821		
0.862	0.870	0.883	0.863	0.841	0.851	0.855			
0.875	0.902	0.911	0.908	0.915	0.931				
0.890	0.918	0.930	0.920						
0.914	0.935	0.942	0.926						
0.928	0.952	0.954							
0.922	0.945								
0.902									

Note: Values in the first row indicate similarities among individuals within each station. The rows that follow indicate similarities between stations of different distances from one another. For example, row four of the 80 m station in the clearcut indicates that individuals sampled at the 80 m and 140 m stations have a band-sharing frequency of 0.925.

tances from one another showed a low frequency of band similarity. This pattern was not seen in the clearcut stream.

Overall, larvae were more genetically similar in the clearcut than in the old growth (Fig. 1). The between-individual mean band-sharing frequency was from 0.921 ± 0.023 to 0.937 ± 0.017 in the clearcut and 0.861 ± 0.018 to 0.889 ± 0.017 in the old growth (Table 4). Larvae 0–160 m apart showed no decrease in genetic relatedness in the clearcut, whereas larvae 0–140 m apart decreased in genetic similarity in the old growth (Fig. 1). Values at 160 and 180 m stream reaches in old growth, and at the 180 m stream reach in the clearcut, (Fig. 1) have high standard errors of the estimate (Table 4) and may represent outliers. Pearson's correlation coefficients revealed a moderate negative correlation between genetic relatedness and physical distance of individuals in the clearcut site ($r = -0.475$, $P = 0.165$), but a high negative correlation between genetic relatedness and physical distance in the old-growth site ($r = -0.786$, $P = 0.007$). The two stream habitats were significantly different in their patterns of gene flow ($P = 0.016$).

Discussion

Heterozygosity

Ascaphus showed significantly lower genetic variation in the clearcut ($H = 0.2275 \pm 0.0259$) than in the old growth ($H = 0.3109 \pm 0.0263$), but the difference in the percentage of polymorphic loci (60.66% and 72.13%, respectively) was

not significant ($\chi^2 = 0.604$). The observed pattern in genetic variation may result from a lower effective population size as a result of clearcut logging. Ritland et al. (2000), also using RAPD data, reported a similar value for mean heterozygosity (0.21 ± 0.02) for *Ascaphus* in a clearcut stream from the same clearcut site. There have been no other studies of local genetic structure for *Ascaphus* with the RAPD technique.

Although it is possible that genetic variation may have been low at the outset owing to few founding members following logging, this seems unlikely because heterozygosity for the nearby old growth is higher. Also, the clearcut was harvested 10 years ago. Because *Ascaphus* have low tolerance to desiccation and temperature extremes (Brattstrom 1963; de Vlaming and Bury 1970; Claussen 1973), few new individuals may have entered the population since logging.

Using pitfall traps operated in three replicates of clearcuts and old-growth forests over 3 years, Wahbe et al. (2004) captured only 33 mature adults in clearcuts compared with 75 mature adults in old-growth forests. Populations in harsher habitats (e.g., clearcuts, urban settings) are expected to show reduced genetic diversity owing to lower effective population sizes and temporary contractions of population size (Ritland et al. 2000).

Population differentiation

Genetic differentiation among reaches within old growth was high ($F_{ST} = 0.3136$). In the clearcut, genetic differentia-

Table 4. Mean frequency of RAPD band sharing among individuals sampled within the same stream-reach station (0 m) and among individuals sampled at increasing distances from one another (20 m apart, 40 m apart, etc.).

Forest cover	Stream-reach distance categories[a]									
	0 m	20 m	40 m	60 m	80 m	100 m	120 m	140 m	160 m	180 m
Clearcut ± SE_E[b]	0.937±0.014	0.936±0.013	0.934±0.014	0.934±0.014	0.935±0.014	0.934±0.015	0.936±0.014	0.936±0.014	0.937±0.017	0.921±0.023
Old growth ± SE_E[b]	0.889±0.017	0.885±0.017	0.882±0.018	0.876±0.017	0.876±0.018	0.868±0.018	0.863±0.017	0.861±0.018	0.864±0.021	0.876±0.026

[a]Distances between stream-reach stations.
[b]Standard errors of the parameter estimates (SE_E) were obtained by bootstrapping, where 100 repeated random samples were selected from the data and the model was estimated from each one (Norusis 1993).

tion was slightly lower (F_{ST} = 0.2285). Lower genetic differentiation among reaches and lower heterozygosity are consistent with a smaller effective population size in the clearcut. We estimated a small F_{ST} value between old growth and clearcut (F_{ST} = 0.0677), indicating a low degree of genetic differentiation among streams. Prior to forest-harvesting activities (10 years ago), adults may have moved between the two streams (1.6 km apart), yielding little differentiation between larvae of the two streams. Taken together, the results suggest a decline in numbers since logging (affirmed by pitfall trapping; Wahbe et al. 2004), which results in a less stable population structure in the clearcut.

In fall, reproductively mature *Ascaphus* congregate at the upper headwaters of streams, and there is evidence that adults have strong site fidelity, which suggests that mating is not random (Daugherty and Sheldon 1982*a*; Wahbe et al. 2004). Moreover, because of the relatively recent and continuing colonization of northern habitats suggested by Ritland et al. (2000), immigration and emigration likely persists. For these reasons, we chose to use AMOVA (Excoffier et al. 1992) as an alternative estimator of population differentiation, because it does not rely on the assumptions of Hardy–Weinberg equilibrium.

AMOVA estimates confirmed that genetic differentiation among old-growth and clearcut streams was small (2.89%; Φ_{ST} = 0.03), and most genetic differentiation (97%) was found within streams. Using AMOVA, Neilson et al. (2001) detected significant genetic differentiation in both coastal (88.7%) and inland (82.7%) populations of *Ascaphus*. Significant differentiation also occurred within a group encompassing 14 populations (Φ_{ST} = 0.571; Neilson et al. 2001). The 6–8 year generation time in *Ascaphus* (hence only one generation since logging in the clearcut) and low metabolic rate may result in a slow evolutionary rate, which can reduce genetic divergence (Martin and Palumbi 1993).

Geographic distance and genetic relatedness

Based on band-sharing frequencies, individuals in the clearcut were more related to one another than were individuals in the old growth (consistent with heterozygosity and F_{ST} estimates). Both streams had more genetically similar individuals at the top of the stream than at the bottom. This observation suggests that larval drift from upper reaches influenced the patterns of genetic relatedness as larvae move farther downstream. Siblings are more likely to be found within close proximity of one another at the upper reaches, closer to where egg masses are deposited. However, in the clearcut, there was a high degree of genetic relatedness among individuals, and geographic distance did not appear to have any influence on relatedness of larvae up to 160 m away. Individuals in old growth, however, showed a decrease in genetic relatedness with increasing geographic distance between sampled reaches. The clearcut population went through a bottleneck/founding event, but also showed greater dispersal. *Ascaphus* were perhaps moving in search of new habitat (which lowers isolation by distance) and were suffering mortality (decreasing diversity).

Our findings may suggest that more movement of larvae across reaches occurs in the clearcut stream, but that is inconsistent with results of Wahbe and Bunnell 2001, who found that *Ascaphus* larvae in clearcut streams moved shorter dis-

Fig. 1. Scatterplot of genetic relatedness (frequency of RAPD band sharing) and physical distance between stream sampling stations (metres apart) for individual *Ascaphus truei* sampled from clearcut and old-growth sites.
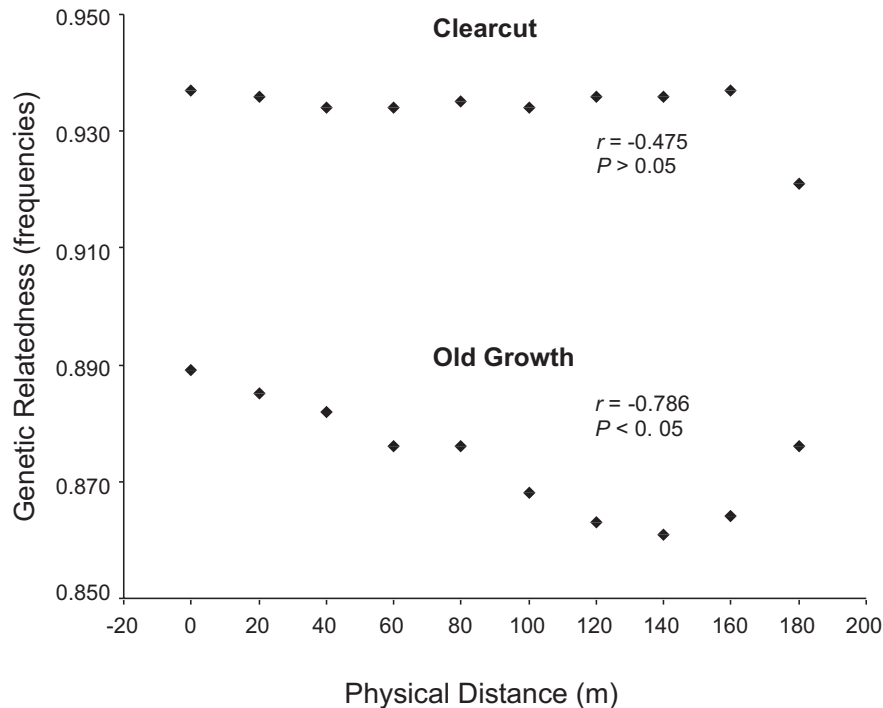
tances (up to 3 m) than those found in old-growth streams (up to 65 m). Stream and site parameters have the potential to influence larval movement within streams, but none appear significantly different to have caused a response in larval movement patterns (see Wahbe and Bunnell 2001). An alternative to more movement is the possibility that many larvae in the clearcut are siblings. The sample may be offspring from as few as two mating pairs, or from closely related parents. Mating pairs that were restricted to the clearcut stream since logging may explain the greater genetic similarity of larvae in the clearcut stream compared with those in the old-growth stream. Differential spatial structures between forest cover types could also be a result of different population sizes.

Conclusions

Most problems in conservation will require genetic and ecological investigations. Our results, despite a lack of replication among streams within different forest cover types, will be important in guiding future research and conservation efforts for *Ascaphus*. Previous ecological research on this species has been helpful in our interpretation of the population genetic results.

There appears to be lower heterozygosity (genetic variation) for larvae captured in the clearcut site, which is consistent with recent demographic data (Wahbe et al. 2004). Heterozygosity can be positively correlated with fitness components such as survival, growth rates, and fertility (Reed and Frankham 2001; Wang et al. 2002; Reed and Frankham 2003). If this is true for *Ascaphus*, our data suggest that individuals in the clearcut are less capable of adapting to environmental threats (e.g., increases in temperature and ultraviolet radiation, pathogenic chytrids, or *Saprolegnia* water molds).

The capture of fewer breeding adults (Wahbe et al. 2004) and the lower genetic variation recorded in the clearcut are consistent with a smaller effective population size. This may mean that populations in the clearcut are less stable (i.e., lower population persistence) than in old growth. Small effective population size and a lack of dispersal between sites may limit mate choice, which leads to breeding with close relatives.

Although data are limited, our results suggest that *Ascaphus* populations exist in a metapopulation structure. Maintenance of connectivity between forested streams will likely be important in allowing for dispersal of *Ascaphus* between populations and in ensuring population persistence. Future investigations should address *Ascaphus* metapopulation structure and extinction risk in fragmented habitats.

Whitlock and McCauley (1999) suggested that movement of individuals is usually more relevant to our understanding of dispersal than gene flow alone. However, direct estimates of dispersal are difficult (Vos et al. 2001), and there are additional limitations to ecological studies such as confounding factors (e.g., watershed, year, developmental stage; see Wahbe et al. 2004). Using ecological or molecular techniques alone to monitor *Ascaphus* populations can be problematic because each technique has limitations. We recommend that any future *Ascaphus* research include both ecological and molecular tools.

Acknowledgements

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