Genetic population structure of the fairy shrimp *Branchinecta coloradensis* (Anostraca) in the Rocky Mountains of Colorado

Andrew J. Bohonak

Abstract: Dispersal rates for freshwater invertebrates are often inferred from population genetic data. Although genetic approaches can indicate the amount of isolation in natural populations, departures from an equilibrium between drift and gene flow often lead to biased gene flow estimates. I investigated the genetic population structure of the pond-dwelling fairy shrimp *Branchinecta coloradensis* in the Rocky Mountains of Colorado, U.S.A., using allozymes. Glaciation in this area and the availability of direct dispersal estimates from previous work permit inferences regarding the relative impacts of history and contemporary gene flow on population structure. Hierarchical *F* statistics were used to quantify differentiation within and between valleys (θ_{SV} and θ_{VT}, respectively). Between valleys separated by 5–10 km, a high degree of differentiation (θ_{VT} = 0.77) corresponds to biologically reasonable gene flow estimates of 0.07 individuals per generation, although it is possible that this value represents founder effects and nonequilibrium conditions. On a local scale (≤110 m), populations are genetically similar (θ_{SV} = 0.13) and gene flow is estimated to be 1.7 individuals exchanged between ponds each generation. This is very close to an ecological estimate of dispersal for *B. coloradensis* via salamanders. Gene flow estimates from previous studies on other Anostraca are also similar on comparable geographic scales. Thus, population structure in *B. coloradensis* appears to be at or near equilibrium on a local scale, and possibly on a regional scale as well.

Résumé : Les taux de dispersion des invertébrés d'eau douce sont souvent évalués à partir des données sur la génétique des populations. Bien que les approches génétiques soient en mesure de nous indiquer l'importance de l'isolement des populations naturelles, des écarts de l'équilibre entre la dérive génétique et le flux génétique aboutissent souvent à des estimations du flux génétique qui sont erronées. J'ai étudié au moyen d'allozymes la structure génétique des populations de *Branchinecta coloradensis*, un anostracé d'eau douce qui vit dans les étangs, dans les montagnes Rocheuses du Colorado, É.-U. Les glaciations dans cette région et la disponibilité d'estimations directes de la dispersion à partir de travaux antérieurs ont permis d'aboutir à des hypothèses sur les impacts relatifs des flux génétiques passés et présents sur la structure des populations. Des statistiques hiérarchiques sur la distribution du rapport des variances ont servi à quantifier la différenciation au sein de chacune des vallées et entre les vallées (θ_{SV} et θ_{VT}, respectivement). Entre des vallées séparées de 5–10 km, un fort degré de différenciation (θ_{VT} = 0,77) corrrespond à une estimation biologiquement raisonnable du flux génétique, soit 0,07 individu par génération, mais il est possible que cette valeur reflète un effet de fondateur ou des conditions de non équilibre. À l'échelle locale, (≤110 m), les populations sont génétiquement semblables (θ_{SV} = 0,13) et le flux génétique est évalué à 1,7 individu échangé entre les étangs chaque génération. Il s'agit là d'une valeur très voisine de l'estimation écologique de la dispersion de *B. coloradensis* par l'intermédiaire de salamandres. Les estimations du flux génétique dans des études antérieures sur d'autres anostracés sont également semblables à des échelles géographiques comparables. La structure de la population de *B. coloradensis* semble donc à l'équilibre ou presque à l'échelle locale, et peut-être aussi à l'échelle régionale.

[Traduit par la Rédaction]

Introduction

For most aquatic invertebrates, direct estimates of dispersal are difficult or impossible to obtain. Freshwater arthropods are typically too small and short-lived to allow standard mark–recapture techniques to be employed and, accordingly, most studies of dispersal in freshwater invertebrates have concentrated on the presumed vectors themselves (e.g., Proctor 1964; Proctor et al. 1967; Peck 1975; Boag 1986) or have inferred dispersal ability from geographic distribution (Hebert and Hann 1986; Brendonck et al. 1990; Saunders et al. 1993). These limitations have led many freshwater biologists to explore genetic population structure as a surrogate for direct estimates of dispersal. Under appropriate conditions, the extent of neutral genetic differentiation among populations can be used to estimate the amount of gene flow, with substantial divergence indicating low dispersal (reviewed by Slatkin 1985). However, because population

Received February 11, 1998. Accepted July 20, 1998.

A.J. Bohonak.[1] Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, NY 14853, U.S.A. and Rocky Mountain Biological Laboratory, P.O. Box 519, Crested Butte, CO 81224, U.S.A.

[1]Present address: Center for Conservation Research and Training, 3050 Maile Way, Gilmore 409, University of Hawaii, Honolulu, HI 96822, U.S.A.

structure is also a consequence of drift, historical and mutational pressures, many scenarios involving different combinations of these factors are almost always possible for any data set. In the Anostraca, for example, significant genetic differences between populations have been interpreted as being a consequence of low dispersal ability (Riddoch et al. 1994) or of recent population expansion, coupled with pronounced founder effects (Boileau et al. 1992). Even in ideal circumstances where populations have reached a genetic equilibrium, gene flow estimates are not expected to translate directly into dispersal. A poor fit between estimates of dispersal and gene flow arises when immigrants do not become part of the breeding population, or when short-term ecological studies do not reflect the long-term averages of gene flow that are inferred from allele frequencies.

For fairy shrimp, population genetic studies have provided conflicting views on the amount of dispersal and gene flow that occur in nature. In a survey of genetic variation covering 15 invertebrate species, Boileau et al. (1992) reported F_{ST} values of 0.075 and 0.360 for Canadian populations of *Artemiopsis stefanssoni* and *Branchinecta paludosa*, corresponding to estimates of 3.1 and 0.4 individuals, respectively, exchanged per generation between populations separated by distances of less than 1 km. However, because gene flow estimates for the 15 species assayed did not correlate with ecological rankings of dispersal potential, Boileau et al. (1992) dismissed these estimates as being unduly influenced by nonequilibrium conditions. Zooplankton populations, they argued, are founded from relatively few individuals and then quickly increase to sizes of 10^4–10^6. In these situations, gene frequencies can take thousands or tens of thousands of generations to reach equilibrium, and the ponds in the study are less than 3000 years old (Boileau et al. 1992). Thus, genetic differentiation in these invertebrates is misleading, and gene flow could actually be one or two orders of magnitude greater than is apparent.

Riddoch et al. (1994) studied genetic structure in populations of *Branchipodopsis wolfi* from southern Africa, which the authors believed to be much older than those in Boileau et al.'s (1992) study. Their estimate of F_{ST} (from 0.064 to 0.076, depending on the method used) translates into 2.4–3.6 individuals exchanged between populations per generation across distances of approximately 10–100 m. This is nearly identical with the value reported for *A. stefanssoni* by Boileau et al. (1992). The concordance suggests that founder effects are not relevant for allozyme studies of North American Anostraca, that the populations in Africa are much younger than believed, or that the three species are so different demographically as to not be comparable. Because there are few quantitative data concerning fairy shrimp dispersal (e.g., Bohonak 1999), it is difficult to reconcile the studies by Boileau et al. (1992) and Riddoch et al. (1994) conclusively.

Recently, Davies et al. (1997) studied allozyme population differentiation in a fourth species of Anostraca, *Branchinecta sandiegonensis* from California, U.S.A. Although the authors do not speculate on the age of these populations, the majority of vernal-pool habitats in that region have been eliminated by agriculture and urbanization over the past century (King et al. 1996). Population differentiation of *B. sandiegonensis* in this fragmented landscape was greater than that found in other fairy shrimp species (F_{ST} = 0.51). Although the large amount of population structure could reflect departures from equilibrium and recent fragmentation of the species' range, *B. sandiegonensis* was sampled on a spatial scale of up to 50 km, much larger than in the other studies.

To determine the accuracy of gene flow estimates in fairy shrimp and other freshwater zooplankton, I assayed allozyme variation in the fairy shrimp *Branchinecta coloradensis* from the Rocky Mountains of Colorado, U.S.A., on both small and regional spatial scales. These populations provide a geological setting unique among genetic studies of Anostraca, permitting broader generalizations regarding the persistence of historical information in measures of population differentiation. In addition, the primary mechanism for small-scale dispersal of *B. coloradensis* has been quantified (salamanders; Bohonak 1999), yielding a minimal expectation for gene flow. Thus, the accuracy of gene flow estimates in this species can be evaluated by comparison with direct dispersal estimates, as well as through contrasts with ecologically similar species in geologically distinct areas.

Materials and methods

Branchinecta coloradensis were collected between 5 and 8 August 1996 from nine ponds in the Elk Mountains of Colorado (Fig. 1). To quantify population structure on scales where different dispersal vectors might act (e.g., salamanders locally and birds regionally; Proctor 1964; Bohonak 1999), clusters of ponds separated by tens of metres were sampled within each of three valleys separated by 5–10 km. Two ponds were sampled at the top of Redwell Basin (RB) and four in Yule Basin (YB). At the Mexican Cut Nature Preserve (MC), two ponds were sampled from the "Lower Cut" (L8 and L18b), and two from the "Upper Cut" (U1 and U2), which is a separate cluster of ponds at an altitude 50 m higher. Additional ponds containing *B. coloradensis* in the MC and YB valleys were not sampled because populations within valleys were assumed a priori to be genetically similar and because the sampling regime was replicated across three independent valleys. Site descriptions for MC are available in Wissinger et al. (1999) and in Wissinger and Whiteman (1992). Pond numbers in the other two valleys correspond to those found by P.L. Nelson.[2] (Site descriptions are also available on request from the author.)

Fairy shrimp were gathered using hand nets and coarse zooplankton nets, transported live to the laboratory within 6 h, and frozen at –80°C. Additional individuals from each population were preserved in ethanol, and taxonomic verification was conducted with Pennak's (1989) key.

Following an initial screen, 19 allozyme loci were assayed electrophoretically, using a cellulose acetate medium with Tris–glycine (TG) and citric acid – aminopropyl morpholine (CAAPM) buffer systems (Table 1). Staining recipes were modified from Hebert and Beaton (1993). Between 37 and 42 fairy shrimp were screened from each pond whenever possible, except for YB3, where only 7 animals were available. An attempt was made to screen all individuals for all loci. All heterozygotes stained according to known quaternary structure (Harris and Hopkinson 1976). Expected and observed (direct count) heterozygosities were calculated for each locus, and exact tests for Hardy–Weinberg equilib-

[2] P.L. Nelson. 1971. Ecology of the fairy shrimp *Branchinecta coloradensis* Packard (Crustacea: Anostraca) related to its distribution in an alpine habitat. B.Sc. thesis, Harvard University, Cambridge, Mass.

Fig. 1. Locations of the three valleys sampled for *Branchinecta coloradensis* in the Elk Mountains of central Colorado. Redwell Basin (RB) and Yule Basin (YB) are located at altitudes of 3420 and 3300 m, respectively. The Mexican Cut Nature Preserve (MC) contains two groups of ponds, one at an altitude of 3400 m and one at 3450 m.

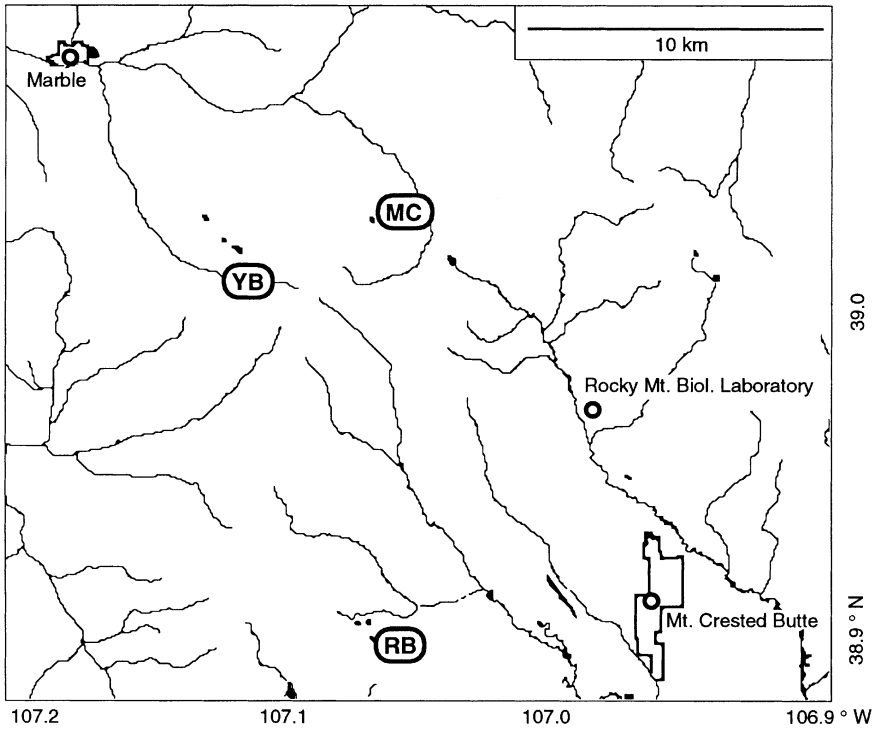


Table 1. Allozymes screened for *B. coloradensis*, electrophoresis running conditions, and the total number of alleles at each locus.

Enzyme	EC No.	No. of loci	Buffer	Run time (min)	No. of alleles
AO	1.2.3.1	1	CAAPM	15	?
AAT	2.6.1.1	2	CAAPM	27	1, 3
ARK	2.7.3.3	1	TG	14	2
FUM	4.2.1.2	1	TG	21	1
GPI	5.3.1.9	1	TG	23	2
G3PDH	1.2.1.12	1	CAAPM	19	1
IDH	1.1.1.42	2	CAAPM	19	1, 1
LDH	1.1.1.27	1	CAAPM	20	1
MDH	1.1.1.37	2	CAAPM	20	3, 1
ME	1.1.1.40	1	CAAPM	22	1
PEP (GL)	3.4.11	1	TG	12	1
PEP (LG)	3.4.11	2	TG	12	1, 1
PGM	2.7.5.1	2	TG	16	1, 4
6PGDH	1.1.1.44	1	CAAPM	20	1

Note: Staining protocol and buffers were modified from Hebert and Beaton (1993).

rium were determined from 500 Monte Carlo simulations for each locus–population combination, using the computer program TFPGA (Miller 1998). To examine the relationship among ponds qualitatively, Nei's genetic distance (*D*) was calculated between all pairs of ponds (Nei 1972). Plots of *D* versus geographic distance were examined, as was an UPGMA (unweighted pair-group method with arithmetic averaging) cluster analysis conducted with TFPGA on genetic distances between the ponds.

For polymorphic loci, hierarchical *F* statistics were calculated from the variance components V_G, V_I, V_S, and V_V for the levels of gene, individual, subpopulation (= pond), and valley, respectively (for an example see the Appendix of Williams and Guries 1995). The mean squares for these components were taken from standard analysis of variance tables for each allele and each locus, using the statistical package JMP (SAS Institute Inc. 1994). Using indicator variables, genes were nested within individuals, individuals within subpopulations, and subpopulations within valleys (Weir 1990). From the mean squares, the *F* statistics F_{IT} (reduction in heterozygosity of the individual relative to the total), f_{IS} (individual to subpopulation), θ_{SV} (subpopulation to valley), and θ_{VT} (valley to total) were calculated following the example of Williams and Guries (1995), who reported formulae for a four-level analysis. This method of determining hierarchical population structure is algebraically identical with the formulae in Weir and Cockerham

Table 2. Gene frequencies, observed heterozygosities (H_O), and number of individuals (n) assayed for polymorphic loci.

		MC				RB		YB			
Locus	Allele	U1	U2	L8	L18b	RB1	RB2	YB3	YB4	YB9	Overall
AAT-2	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.002
	2	0.00	0.01	0.00	0.00	0.00	0.03	0.50	0.85	0.80	0.223
	3	1.00	0.99	1.00	1.00	1.00	0.97	0.50	0.15	0.19	0.775
	H_O	0.000	0.027	0.000	0.000	0.000	0.051	0.429	0.250	0.310	0.119
	n	40	37	40	40	40	39	7	40	42	325
ARK	1	0.00	0.00	0.06	0.10	0.00	0.00	0.00	0.00	0.00	0.020
	2	1.00	1.00	0.94	0.90	1.00	1.00	1.00	1.00	1.00	0.980
	H_O	0.000	0.000	0.075	0.200	0.000	0.000	0.000	0.000	0.000	0.031
	n	40	37	40	40	39	40	7	40	42	325
GPI	1	1.00	1.00	1.00	0.91	1.00	1.00	1.00	1.00	1.00	0.913
	2	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.088
	H_O	0.000	0.000	0.000	0.175	0.00	0.000	0.000	0.000	0.000	0.019
	n	40	37	40	40	40	40	7	40	42	326
MDH-1	1	0.76	0.99	1.00	1.00	0.84	0.97	0.93	1.00	0.99	0.943
	2	0.00	0.00	0.00	0.00	0.16	0.03	0.07	0.00	0.01	0.026
	3	0.24	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.031
	H_O	0.425	0.028	0.000	0.000	0.075	0.051	0.143	0.000	0.024	0.083
	n	40	36	38	40	40	39	7	40	42	322
PGM-2	1	0.00	0.01	0.00	0.00	0.00	0.00	1.00	1.00	1.00	0.275
	2	0.16	0.30	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.057
	3	0.83	0.69	0.98	1.00	1.00	1.00	0.00	0.00	0.00	0.667
	4	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.002
	H_O	0.200	0.351	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.061
	n	40	37	40	40	40	40	7	40	42	326

Note: All loci with two or more alleles were used in the analyses. Alleles at each locus were numbered according to their mobility, with allele 1 being the slowest. Overall heterozygosities for each locus are averaged by subpopulation. MC: Mexican Cut Nature Preserve; U1, U2, L8, and L18b: ponds (= subpopulations) in MC; RB: Redwell Basin; RB1 and RB2: ponds (= subpopulations) in Redwell Basin; YB: Yule Basin; YB3, YB4, and YB9: ponds (= subpopulations) in YB.

(1984) and Weir (1990). To estimate variance with methods comparable to those in previous studies, 95% confidence intervals for the components of population structure were determined by jackknifing across alleles, as well as from the center of 500 bootstraps conducted across loci. For inferences made at the level of the valley, the F statistics were also jackknifed across the three valleys.

Results

Of the 19 allozyme loci that could be reliably scored, 13 were monomorphic and 1 (AO) was discarded, owing to uninterpretable patterns of variation (Table 1). Uninterpretable patterns of variation in AO have been noted previously in other crustacean zooplankton (De Melo and Hebert 1994). Allele frequencies and heterozygosities for the 5 polymorphic loci are shown in Table 2. Using exact p values generated from 10 000 permutations per test (Miller 1998), two significant departures from Hardy–Weinberg equilibrium were detected at $p < 0.05$ that were due to heterozygote deficiencies (MDH-1 from subpopulation RB1 and PGM-2 from subpopulation L8). After Bonferroni correction for multiple tests, only the MDH-1 locus from RB1 departed significantly from Hardy–Weinberg equilibrium ($p = 0.0017$). The observed heterozygosity (H_O) averaged across the 5 polymorphic loci was 0.063, and H_O was 0.017 for all 18 scorable loci.

Significant differentiation was found at all levels of the hierarchical analysis, and the jackknifed standard errors associated with these estimates were generally low (Table 3). Despite only one significant departure from Hardy–Weinberg equilibrium, the inbreeding coefficient (f_{IS}) was calculated to be 0.16, suggesting that ponds are not composed of panmictic breeding pools. In four of the five polymorphic loci, f_{IS} was greater than zero (Table 4), although at each locus, heterozygote deficiencies were only found in one or two populations. For example, the high estimate of f_{IS} for PGM-2 was due to a single individual homozygous for a rare allele in the pond L8 subpopulation and a nonsignificant heterozygote deficiency in the pond U1 subpopulation ($p = 0.07$ for departure from Hardy–Weinberg equilibrium). At MDH-1, only the pond RB1 subpopulation showed a deficiency of heterozygotes ($p = 4.6 \times 10^{-6}$). Contrasting levels of significance between f_{IS} and departures from Hardy–Weinberg equilibrium can occur if the heterozygote deficiencies are not large, because statistical tests of Hardy–Weinberg equilibrium are notoriously weak.

Jackknifed estimates of f_{IS}, θ_{SV}, and θ_{VT} were slightly higher than the direct estimates, and confidence intervals from bootstrapping across loci were larger than the jackknifed confidence intervals. For the estimate of population differentiation between valleys, the bootstrapped variance was extremely high, because although no single locus markedly biased θ_{VT}, there was a high degree of variation

Table 3. Hierarchical population structure in *B. coloradensis* calculated from the five polymorphic loci directly (θ), by jackknifing across loci ($\theta_{jackknife-loci}$), and by jackknifing over valleys (Weir 1990).

	f_{IS}	θ_{SV}	Nm_{SV}[a]	θ_{VT}	Nm_{VT}[a]
Method					
θ	0.158	0.124	1.77	0.714	0.10
$\theta_{jackknife-loci}$ (mean ± 1 SE)	0.163±0.028	0.127±0.014	1.72	0.774±0.059	0.07
95% CI calculated from:					
jackknife variances across loci	0.107–0.219	0.098–0.155	1.36–2.29	0.658–0.891	0.03–0.13
bootstrapping across loci	0.043–0.260	0.059–0.165	1.27–3.97	–0.024 to 0.705	0.10–∞
jackknife variances over valleys	0.048–0.217	0.009–0.101	2.22–27.79	0.001–1.214	0–408.03

[a]Assuming equilibrium, the number of migrants per generation (*Nm*) was derived from $Nm = (\theta^{-1} - 1)/4$.

Table 4. Locus-specific variance components and estimates of population structure.

	Variance				Population statistic			
	V_G	V_I	V_S	V_V	F_{IT}	f_{IS}	θ_{SV}	θ_{VT}
AAT-2	0.089	0.006	0.006	0.190	0.693	0.060	0.057	0.654
ARK	0.034	0.003	0.002	0.000	0.141	0.089	0.055	0.002
GPI	0.021	–0.002	0.002	0.000	–0.009	–0.082	0.089	–0.023
MDH-1	0.078	0.018	0.018	–0.003	0.296	0.187	0.158	–0.028
PGM-2	0.064	0.029	0.020	0.349	0.861	0.308	0.179	0.755

Fig. 2. Nei's *D* as a function of geographic distance for all pairs of populations.

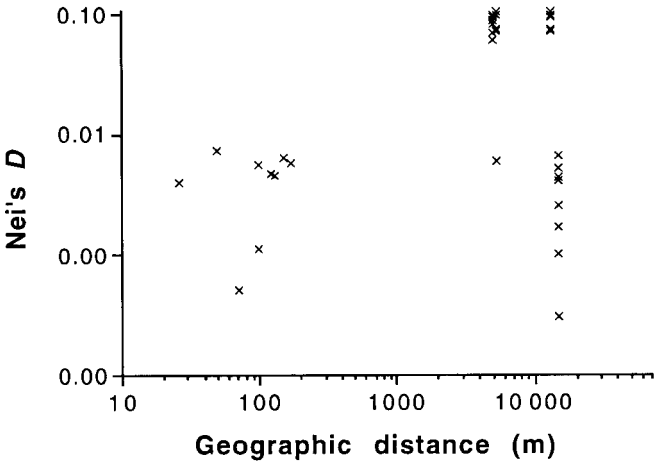
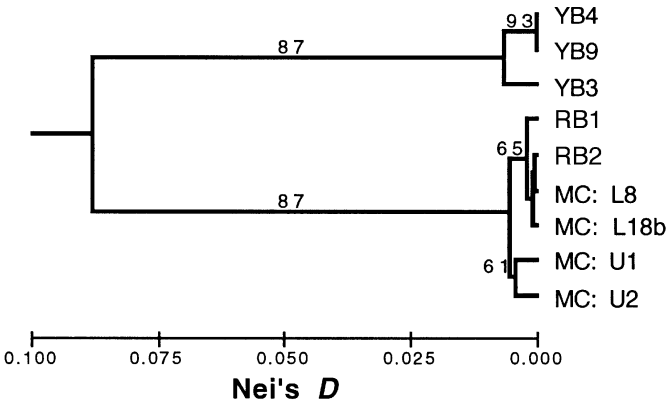


Fig. 3. UPGMA dendrogram based on Nei's *D*. Bootstrap support is indicated above groups with more than 50% support (500 permutations; Miller 1998).



among loci (Table 4). Estimates of θ_{VT} that were jackknifed across valleys also showed a large amount of variation, corresponding to the distribution of Nei's *D* (Figs. 2 and 3). If equilibrium in the system is assumed, the values of θ_{SV} = 0.127 and θ_{VT} = 0.774 translate to an average of 1.7 individuals exchanged between subpopulations in the same valley each generation, and one individual exchanged between valleys every 14.3 generations.

The average value of Nei's *D* between all pairs of subpopulations was 0.043. A UPGMA dendrogram revealed that the YB pond subpopulations were genetically distinct from the remainder of the subpopulations, but relatively short genetic distances separated the MC and RB pond subpopulations and they did not cluster entirely by valley (Figs. 2 and 3). The genetic distinctness of the subpopulations in YB is due to the fixation of allele 1 at PGM-2, which is absent from five of the other six populations, and to the reduced frequency of allele 3 at AAT-2 (Table 2). Although the subpopulations in the Upper Cut ponds (U1 and U2) in MC formed a distinct and statistically significant cluster, the nearby subpopulations of the Lower Cut ponds (L8 and L18b) were more similar to subpopulations in RB than to the those in the Upper Cut. This can be seen in Table 2: there are no large differences in allele frequencies between the subpopulations of the MC and RB valleys.

The failure of populations to cluster entirely by valley in the phenetic analysis (Fig. 2) might indicate that it is inappropriate to use this scale in a hierarchical analysis of population structure. However, several lines of reasoning suggest that this is not the case. First, one allele that was unique to the Upper and Lower Cuts and absent from RB was found (PGM-2: 2) and, in general, Nei's *D* was greater for between-valley comparisons than for within-valley comparisons (Mann–Whitney *U* test statistic = 211, U' = 49, p = 0.0033; see Figs. 2 and 3). Further, the amount of differentiation

within the MC and RB subpopulations was very small compared with the differences between these valleys taken together and YB. The L8–L18b–RB cluster had weak bootstrap support that was dependent entirely on the PGM-2 locus (Table 2, Fig. 3).

The most conservative approach is to analyze these populations on the basis of their geographic distributions and their presumed accessibility to dispersal. Ponds in this mountainous region have distributions that are naturally disjunct, and rates of dispersal are likely to be much higher within valleys than between them. Other alternatives include conducting three to four separate analyses, promoting the Upper and Lower Cut pond groups to a hierarchical level equivalent to the RB and YB valleys, or analyzing all ponds with a single estimate of F_{ST}. The last two options do not lead to qualitatively different conclusions regarding gene flow in *B. coloradensis*. A single island model yields a *Nm* estimate of 0.12 ($\theta = 0.68$), and if the Upper and Lower Cut ponds are treated as "valleys," *Nm* within valleys is estimated to be 3.3 ($\theta_{SV} = 0.07$) and *Nm* between valleys to be 0.16 ($\theta_{VT} = 0.61$). The addition of new genetic markers and more valleys would better resolve this ambiguity.

Discussion

Levels of allozyme variation are low in *B. coloradensis*, with only 28% of the loci possessing more than one allele, and average $H_O = 0.017$. Although neither Boileau et al. (1992) nor Riddoch et al. (1994) reported these summary statistics, variation in this species is lower than that reported for other freshwater crustacean zooplankton (De Melo and Hebert 1994; Hann 1995) and much lower than values previously reported for the anostracan *Artemia* (Gajardo et al. 1995). Low heterozygosity in these populations of *B. coloradensis* is consistent with a relatively recent origin for these populations from unglaciated populations at lower elevations. *Branchinecta coloradensis* is usually an alpine species, but has been found at low elevations in Colorado and throughout much of western North America (Gordon 1932; Dexter 1959). Although brief population bottlenecks alone do not dictate reductions in heterozygosity (e.g., Balanya et al. 1994), a loss of variation would be seen if local range expansion in this species was recent and the number of founders for each new population was small (e.g., Fleischer et al. 1991). However, unusually low levels of variation may not affect quantitative interpretations of *F* statistics, because population differentiation reaches equilibrium more quickly than heterozygosity (Slatkin 1994).

The estimate of approximately 1–3 individuals exchanged within clusters of ponds each generation (Table 3) is remarkably close to an independent ecological assessment of dispersal via salamanders for MC (Bohonak 1999). Metamorphic adult salamanders in this population feed on fairy shrimp and occasionally migrate with full guts between ponds. Over a 7-year period, an average of 35 viable *B. coloradensis* eggs were estimated to be moved annually by salamanders (*Ambystoma tigrinum nebulosum*) between ponds. Because some of these eggs can survive digestion, approximately 2.7–3.9 individuals are exchanged per generation between ponds (Bohonak 1999). Unless unknown vectors are dispersing at least as many eggs as the salamanders, the correspondence between gene flow estimates and ecologically obtained dispersal estimates suggests that extreme departures from equilibrium (sensu Boileau et al. 1992) do not occur on a local scale. However, information regarding other dispersal vectors (e.g., wind, overland flow, other vertebrates) is largely anecdotal. Waterfowl are uncommon in these high-altitude ponds and birds have not been observed feeding on fairy shrimp. A few temporary ponds in these systems overflow following annual snowmelt, and some fairy shrimp eggs or nauplii may be dispersed in this way.

Equilibrium within valleys is not necessarily indicative of equilibrium between valleys. The time required to reach equilibrium in simple island models is determined by the inverse of the per capita migration rate for large N_e (Slatkin 1994); for example, 5000 – 10 000 generations is required for $N_e = 1000$ and $m = 0.0001$. However, if populations are small or migration rates high, the time can be much less (e.g., for $N_e = 100$ and $m = 0.05$, equilibrium can be reached in 50–100 generations). Thus, equilibrium will be reached between local populations before it is reached regionally if migration rates decrease with distance.

Nonetheless, it seems unlikely that *B. coloradensis* populations depart from equilibrium even on a regional scale. Unlike the very large populations (10^4–10^6) and short time periods (<3000 years) considered in the models of Boileau et al. (1992), only 20–1000 mature adult *B. coloradensis* are typically observed in any population, and glaciation in this area of Colorado ended 10 000 years ago (Prather 1982). Iterative calculations of F_{ST} for an island model show that extreme combinations of N_e and m must be invoked to make even moderate departures from equilibrium likely (A.J. Bohonak, unpublished data). For example, equilibrium is reached in fewer than 2000 generations unless $N_e > 300$ and $m < 1 \times 10^{-10}$, and departures from equilibrium only extend to 4000 or more generations when $N_e > 500$. Although simulations under more realistic conditions are necessary to make firm quantitative conclusions, it is clear that an estimate of 1 individual exchanged every 14 generations for valleys separated by 5–10 km is theoretically plausible as well as biologically reasonable.

Because *B. coloradensis* is obligately sexual, the significant nonzero value obtained for f_{IS} suggests that subpopulations are structured in some manner that prevents panmixis. Although f_{IS} was not calculated by Boileau et al. (1992) or Riddoch et al. (1994), Davies et al. (1997) estimated $f_{IS} = 0.276$ for *B. sandiegonensis*. One explanation for these nonzero f_{IS} values would be assortative mating based on unknown morphological or biochemical criteria. Although a second interpretation might be made in terms of Wahlund effects (spatial structure within subpopulations), the swimming ability of these zooplankters and the small size of the ponds in which they live (<1 m^2 – 500 m^2) make this unlikely. A third possibility is that in some of the smallest ponds, very few individuals successfully breed each year (≈50 or less). In this case, genetic drift would be relatively high between years. However, *B. coloradensis* produces diapausing eggs, and if less than 100% of these eggs hatch annually, a temporally structured pool of propagules would exist in the pond sediments. Even if the propagules from each individual year are in Hardy–Weinberg equilibrium, the annual hatching of eggs from many different years would

yield f_{IS} values greater than zero in the juveniles and adults. Long-lived "egg banks" have been described for other freshwater zooplankton (De Stasio 1989; Hairston et al. 1995; Cáceres 1998) and are probably a widespread phenomenon (Hairston 1996; Hairston and Cáceres 1996).

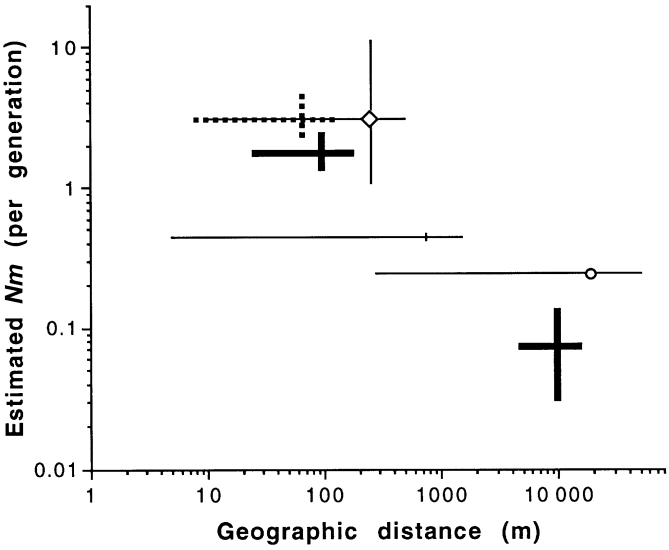
The influence of a long-lived egg bank on generation time may also confound quantitative interpretations of gene flow in *B. coloradensis* and other zooplankton. The assumption that generation times in temperate systems are equal to 1 year is unlikely to be correct in some species that produce diapausing eggs. Invertebrate egg banks in lakes can store viable propagules for dozens or even hundreds of years (Hairston et al. 1995; Hairston 1996; Cáceres 1998), although no estimates of the age of diapausing eggs are available for temporary-pond species. Thus, estimates of 2 individuals exchanged per generation may seem less reasonable, if the realized generation time for anostracans is actually 10 years or more. Further, hatching strategies are expected to covary with habitat predictability (Cohen 1966; Ellner 1985), which could be different in regions such as North America and Africa (where *B. wolfi* was sampled). If this was true, comparing gene flow estimates for zooplankton in different regions would be difficult. Studies that elucidate the ways in which overlapping generations confound simple interpretations of population structure in both single- and multi-species studies of freshwater invertebrates are needed.

Comparisons with other Anostraca

Comparisons of the population structure in *B. coloradensis* with that of other ecologically, phylogenetically, or geographically similar Anostraca can be informative if they share a common history (e.g., Avise 1992) or differ markedly in dispersal ability (e.g., Waples 1987). The conclusions of Boileau et al. (1992) regarding Canadian populations of *A. stefanssoni* and *B. paludosa* were based in part on comparisons with sympatric taxa. Ecological rankings of dispersal for 15 taxa in the study (ranging from Anostraca to Cladocera to Collembola) did not correlate with gene flow estimates. To independently test their hypothesis regarding extreme founder effects, it would be informative to know if older zooplankton populations are less differentiated than younger populations, as gene flow should erode differences between populations over time. As discussed above, a maximum age of 10 000 years is expected for the *B. coloradensis* populations in this study. The *B. wolfi* populations studied by Riddoch et al. (1994) lie on granite outcrops near the edge of the Kalahari desert. The authors do not estimate an age for these ponds, but they are probably much older than the ponds in this study or in that of Boileau et al. (1992). Although glaciation elsewhere presumably affected precipitation and temperature patterns in that area of Africa, it was not glaciated during the Quarternary period (Haughton 1969).

Based on the regional history of existing fairy shrimp studies, one would expect differentiation to increase in the order *B. wolfi* < *B. coloradensis* < *A. stefanssoni* and *B. paludosa*, if founder effects occur in all freshwater anostracans and the age of the populations decreases in that order. Davies et al. (1997) do not speculate on the age of the *B. sandiegonensis* populations; however, their discussion of extensive habitat fragmentation suggests that this species may be far from a drift – gene flow equilibrium.

Fig. 4. Estimates of gene flow in the fairy shrimp *B. coloradensis* (thick lines: this study), *A. stefanssoni* (◇; Boileau et al. 1992), *B. paludosa* (thin lines, no symbol; Boileau et al. 1992), *B. wolfi* (broken line; Riddoch et al. 1994), and *B. sandiegonensis* (○; Davies et al. 1997) as a function of the geographic distance between populations. Minimum, average, and maximum distances between pairs of populations are presented for each species. For *B. coloradensis* and *B. wolfi*, *Nm* values represent jackknifed estimates and 95% confidence intervals based on jackknife variances. Confidence intervals could not be determined for *B. sandiegonensis*. For *A. stefanssoni* and *B. paludosa*, central estimates are derived from two-locus G_{ST} values (Nei 1977), and extreme values are derived from the two single-locus estimates available for each species.



To address this question, in Fig. 4 the estimated numbers of migrants per generation (*Nm*) are plotted for *A. stefanssoni*, *B. coloradensis*, *B. paludosa*, *B. sandiegonensis*, and *B. wolfi* as a function of geographic scale. Slatkin (1993, 1994) recommends log–log plots of this type for pairs of populations as a way of detecting patterns of gene flow even before equilibrium has been achieved, and their use has now become commonplace (e.g., Lynch and Spitze 1994; Peterson 1995; Hellberg 1996). Two patterns are apparent from Fig. 4. First, gene flow estimates in Anostraca decrease as a function of geographic distance in what appears to be a linear fashion on logarithmic axes. This is consistent with reduced amounts of dispersal in a system at equilibrium, but is also consistent with the slower erosion of founder effects as gene flow decreases. More importantly, gene flow estimates on local scales are remarkably similar in three of the four species, at approximately 1–3 individuals per generation, and in the fourth (*B. paludosa*) is within an order of magnitude, at 1 individual every 2 generations (although *B. paludosa* was sampled on a slightly larger geographic scale).

One conclusion to be drawn from this concordance might be that the rate of approach to equilibrium is very low, even compared with the age of the *B. wolfi* ponds in Africa, and

that F_{ST} estimates of ≈0.1 are to be expected on a small scale based on founder events common to all Anostraca. Thus, actual gene flow could be much higher than inferred. Alternatively, populations of all species may be very young and far from equilibrium. Finally, it is possible that all of these Anostraca are at or near equilibrium, and that 1–3 propagules exchanged per generation is reasonable for fairy shrimp in small clusters of temporary ponds. Figure 2 seems to support the latter hypothesis, as there is no reason to believe that the extreme influence of founder events should produce a pattern of isolation by distance.

Inferences made from these cross-species comparisons should be qualified in light of the limitations of the individual studies. For the *B. coloradensis* populations studied here, uneven differentiation among valleys could be consistent with large-scale departures from equilibrium. (However, because of the stochastic nature of the drift process, the analysis of three separate small-scale clusters could be considered an advantage.) Figure 4 shows that the gene flow estimates for *A. stefanssoni* and *B. paludosa* are somewhat different despite their presumed common geological history. (However, it is possible that the histories of these two species are not identical, as they were not collected sympatrically.) Further, only 2 polymorphic loci were found for these species (Boileau et al. 1992). For *B. wolfi*, 5 polymorphic loci were analyzed, but only for 7 ponds within a single locale (Riddoch et al. 1994). Finally, *B. sandiegonensis* exists in a disturbed landscape, with most of its historically present habitat now gone (Davies et al. 1997). It is also possible that natural vectors for dispersal of *B. sandiegonensis* have been eliminated or superceded by anthropogenic ones. Thus, these five species may not be comparable ecologically or in terms of geological history, and the gene flow estimates from each study probably differ in their precision. However, the consistency in genetic population structure found in Anostraca despite their many differences is intriguing enough to warrant further work on new species in new regions.

Acknowledgments

I thank Nelson Hairston, Jr., Richard Harrison, Rick Williams, and two anonymous reviewers for comments, Carla Cáceres for help in the field, and Sam Flaxman for laboratory assistance. The Rocky Mountain Biological Laboratory (RMBL) and The Nature Conservancy (TNC) graciously permitted use of the Mexican Cut Nature Preserve. I am grateful to TNC, the Snyder Memorial Fund of RMBL, the Theodore Roosevelt Memorial Fund (American Museum of Natural History), Sigma Xi, and the National Science Foundation (DEB-9423603 and a training grant to Cornell University's Center for Applied Mathematics and Department of Ecology and Systematics) for funding.

References

Avise, J.C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos, **63**: 62–76.

Balanya, J., Segarra, C., Prevosti, A., and Serra, L. 1994. Colonization of America by *Drosophila subobscura*: the founder event and a rapid expansion. J. Hered. **85**: 427–432.

Boag, D.A. 1986. Dispersal in pond snails: potential role of waterfowl. Can. J. Zool. **64**: 904–909.

Bohonak, A.J., and Whiteman, H.H. 1999. Dispersal of the fairy shrimp *Branchinecta coloradensis* Anostraca): effects of hydroperiod and salamanders. Limnol. Oceanogr. **44**. In press.

Boileau, M.G., Hebert, P.D.N., and Schwartz, S.S. 1992. Nonequilibrium gene frequency divergence: persistent founder effects in natural populations. J. Evol. Biol. **5**: 25–39.

Brendonck, L., Thiery, A., and Coomans, A. 1990. Taxonomy and biogeography of the Galapagos branchiopod fauna (Anostraca, Notostraca, Spinicaudata). J. Crustacean Biol. **10**: 676–694.

Cáceres, C.E. 1998. Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. Ecology, **79**: 1699–1710.

Cohen, D. 1966. Optimizing reproduction in a randomly varying environment. J. Theor. Biol. **12**: 119–129.

Davies, C.P., Simovich, M.A., and Hathaway, S.A. 1997. Population genetic structure of a California endemic branchiopod, *Branchinecta sandiegonensis*. Hydrobiologia, **359**: 149–158.

De Melo, R., and Hebert, P.D.N. 1994. Allozymic variation and species diversity in North American Bosminidae. Can. J. Fish. Aquat. Sci. **51**: 873–880.

De Stasio, B.T., Jr. 1989. The seed bank of a freshwater crustacean: copepodology for the plant ecologist. Ecology, **70**: 1377–1389.

Dexter, R.W. 1959. Anostraca. *In* Fresh-water biology. *Edited by* H.B. Ward and G.C. Whipple. John Wiley and Sons, New York. pp. 558–571.

Ellner, S. 1985. ESS germination strategies in randomly varying environments. II. Reciprocal yield law models. Theor. Popul. Biol. **28**: 80–116.

Fleischer, R.C., Conant, S., and Morin, M.P. 1991. Genetic variation in native and translocated populations of the Laysan finch *Telespiza cantans*. Heredity, **66**: 125–130.

Gajardo, G., Da Conceicao, M., Weber, L., and Beardmore, J.A. 1995. Genetic variability and interpopulational differentiation of *Artemia* strains from South America. Hydrobiologia, **302**: 21–29.

Gordon, K. 1932. *Branchinecta coloradensis* in Colorado. Science (Washington, D.C.), **75**: 287–288.

Hairston, N.G., Jr. 1996. Zooplankton egg banks as biotic reservoirs in changing environments. Limnol. Oceanogr. **41**: 1087–1092.

Hairston, N.G., Jr., and Cáceres, C.E. 1996. Distribution of crustacean diapause: micro- and macroevolutionary pattern and process. Hydrobiologia, **320**: 27–44.

Hairston, N.G., Jr., Van Brunt, R.A., Kearns, C.M., and Engstrom, D.R. 1995. Age and survivorship of diapausing eggs in a sediment egg bank. Ecology, **76**: 1706–1711.

Hann, B.J. 1995. Genetic variation in *Simocephalus* (Anomopoda: Daphniidae) in North America: patterns and consequences. Hydrobiologia, **307**: 9–14.

Harris, H., and Hopkinson, D.A. 1976. Handbook of enzyme electrophoresis in human genetics. American Elsevier, New York.

Haughton, S.H. 1969. Geological history of southern Africa. Geological Society of South Africa, Cape Town.

Hebert, P.D.N., and Beaton, M.J. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis: a practical handbook. Rev. ed. Helena Laboratories, Beaumont, Texas.

Hebert, P.D.N., and Hann, B.J. 1986. Patterns in the composition of Arctic tundra pond microcrustacean communities. Can. J. Fish. Aquat. Sci. **43**: 1416–1425.

Hellberg, M.E. 1996. Dependence of gene flow on geographic distance in two solitary corals with different larval dispersal capabilities. Evolution, **50**: 1167–1175.

King, J.L., Simovich, M.A., and Brusca, R.C. 1996. Species richness, endemism, and ecology of crustacean assemblages in northern California vernal pools. Hydrobiologia, **328**: 85–116.

Lynch, M., and Spitze, K. 1994. Evolutionary genetics of *Daphnia*. *In* Ecological genetics. *Edited by* L.A. Real. Princeton University Press, Princeton, N.J. pp. 109–128.

Miller, M.P. 1998. TFPGA: tools for population genetic analyses for Windows. University of Arizona, Flagstaff.

Nei, M. 1972. Genetic distance between populations. Am. Nat. **106**: 283–292.

Nei, M. 1977. *F*-statistics and analysis of gene diversity in subdivided populations. Ann. Hum. Genet. **41**: 225–233.

Peck, S.B. 1975. Amphipod dispersal in the fur of aquatic mammals. Can. Field-Nat. **89**: 181–182.

Pennak, R.W. 1989. Fresh-water invertebrates of the United States. 3rd ed. John Wiley and Sons, New York.

Peterson, M.A. 1995. Phenological isolation, gene flow and developmental differences among low- and high-elevation populations of *Euphilotes enoptes* (Lepidoptera: Lycaenidae). Evolution, **49**: 446–455.

Prather, T. 1982. Geology of the Gunnison country. B and B Printers, Gunnison, Inc., Gunnison, Colo.

Proctor, V.W. 1964. Viability of crustacean eggs removed from ducks. Ecology, **45**: 656–658.

Proctor, V.W., Malone, C.R., and DeVlaming, V.L. 1967. Dispersal of aquatic organisms: viability of disseminules recovered from the intestinal tract of captive killdeer. Ecology, **48**: 672–676.

Riddoch, B.J., Mpoloka, S.W., and Cantrell, M. 1994. Genetic variation and localized gene flow in the fairy shrimp, *Branchipodopsis wolfi*, in temporary rainwater pools in southeastern Botswana. *In* Genetics and evolution of aquatic organisms. *Edited by* A.R. Beaumont. Chapman and Hall, New York. pp. 96–102.

SAS Institute Inc. 1994. JMP statistics and graphics guide for Macintosh. SAS Institute Inc., Cary, N.C.

Saunders, J.F., III, Belk, D., and Dufford, R. 1993. Persistence of *Branchinecta paludosa* (Anostraca) in southern Wyoming, with notes on zoogeography. J. Crustacean Biol. **13**: 184–189.

Slatkin, M. 1985. Gene flow in natural populations. Annu. Rev. Ecol. Syst. **16**: 393–430.

Slatkin, M. 1993. Isolation by distance in equilibrium and nonequilibrium populations. Evolution, **47**: 264–279.

Slatkin, M. 1994. Gene flow and population structure. *In* Ecological genetics. *Edited by* L.A. Real. Princeton University Press, Princeton, N.J. pp. 3–17.

Waples, R.S. 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution, **41**: 385–400.

Weir, B.S. 1990. Genetic data analysis: methods for discrete population analysis. Sinauer Associates, Sunderland, Mass.

Weir, B.S., and Cockerham, C.C. 1984. Estimating F statistics for the analysis of population structure. Evolution, **38**: 1358–1370.

Williams, C.F., and Guries, R.P. 1995. Genetic consequences of seed dispersal in three sympatric forest herbs. I. Hierarchical population-genetic structure. Evolution, **48**: 791–805.

Wissinger, S.A., and Whiteman, H.H. 1992. Fluctuation in a Rocky Mountain population of salamanders: anthropogenic acidification or natural variation? J. Herpetol. **26**: 377–391.

Wissinger, S.A., Bohonak, A.J., Whiteman, H.H., and Brown, W.S. 1999. Subalpine wetlands in central Colorado: habitat permanence, salamander predation and invertebrate communities. Chapt. 32. *In* Invertebrates in freshwater wetlands of North America: ecology and management. *Edited by* D.P. Batzer, R.R. Rader, and S.A. Wissinger. John Wiley and Sons, New York. In press.