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Effect of insect-mediated dispersal on the genetic structure of postglacial water mite populations

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Assaying population structure in species that differ in dispersal ability can help to determine whether population differentiation is dependent on the movement of individuals between populations. Here, allozyme variation is analysed in over 1100 individuals from nine species and two species complexes of *Arrenurus* water mites collected throughout north-eastern North America. As larvae, eight taxa are obligate parasites of winged adult insects that provide the primary opportunity for dispersal. Three additional species have lost the ability to parasitize insects and do not disperse in this manner. Consistent with the glaciated history of the region, very low allozyme heterozygosity was found in these taxa (H_o = 0.00–0.12), near panmixia in five out of seven species for which population differentiation was calculated and no patterns of isolation by distance over spatial scales up to several hundred kilometres. Nonetheless, in two out of three comparisons between sister species with and without parasitic larvae, parasitism was significantly associated with higher heterozygosity. Population differentiation could also be contrasted for two of these sister species pairs; in each case, lower estimates of F_{ST} were found in the mites able to disperse on insects. The statistical significance of these contrasts was dependent on the method used to estimate variance. At the scale of the genus, behavioural differences among insect vectors allows for broader hypotheses that relate water mite genetic diversity to dispersal ability. For the genus, rank correlations of dispersal ability with direct count heterozygosity (n = 11) and population differentiation (n = 7) were not significantly different from zero. These results are consistent with the hypothesis that allozyme population structure is primarily the result of historical patterns in these regions. However, comparisons between sister species suggest a limited role for dispersal in homogenizing populations genetically, even when drift–gene flow equilibrium has not been achieved.

Keywords: Acari, allozymes, freshwater invertebrate, F_{ST}, gene flow, population genetics.

Introduction

The relationships among dispersal, gene flow and genetic population differentiation have remained resistant to generalizations despite a continued accumulation of studies over the past three decades. The use of allozymes for population genetic studies has been particularly controversial. Although allozyme population differentiation is often used to make inferences regarding the movement of individuals, some have characterized allozyme differentiation as having little or nothing to do with ongoing gene flow, because of historical influences (e.g. Boileau *et al.*, 1992; Bossart & Prowell, 1998) or pervasive natural selection (Karl & Avise, 1992). One way to separate these factors is to assay loci that are likely to evolve at different rates and under different selective regimes (e.g. Burton & Lee, 1994). An alternative is to determine the relative effects of factors such as history, natural selection and drift–gene flow balance using comparative studies focused on multiple species. For example, geographical patterns in mtDNA lineages show a common break between Atlantic and Gulf Coast populations on the east coast of North America in many taxa, concordant with the geological history of the region (Avise, 1992). Other comparative studies have focused on species that differ in dispersal ability, and these have found that species

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with greater dispersal abilities generally show less population differentiation than species with restricted dispersal (e.g. Waples, 1987; reviewed by Bohonak, 1999).

Comparative tests of hypotheses that link dispersal ability to genetic differentiation have often concentrated on marine species differing in larval development and planktonic duration, because species with planktotrophic larvae are hypothesized to disperse further than species with nonplanktotrophic larvae (see Hedgecock, 1986; Palumbi, 1995). However, studies comparing genetic population structure in nonmarine species that differ in dispersal potential often include distantly related taxa. This can bias the results of comparative studies towards the conclusion that ongoing dispersal is unrelated to population structure. As differences in phylogenetic history, ecology and biogeography increase, confounding factors will increasingly weaken correlations and muddle interpretation of the results. The ideal model system would include multiple sympatric sister species differing only in their ability to disperse, and these types of taxa will provide the strongest evaluation of the relationship between dispersal and population differentiation.

Organisms living in recently glaciated areas of North America provide a particularly demanding system in which to test correlations between dispersal, gene flow and population differentiation. Freshwater invertebrates in this region have been characterized as being far from equilibrium because of extreme founder events and recent glaciation (e.g. Hebert & Hann, 1986), and allozyme-based gene flow estimates in these animals seem to bear no relationship to the amount of dispersal that is occurring (Boileau *et al*., 1992). However, the conclusions drawn by Boileau *et al*. (1992) were based on comparisons between species from different classes and phyla. Here, the relationship between dispersal and population differentiation in north-eastern North America has been assessed for a group of freshwater invertebrates more taxonomically restricted than those studied to date. Larvae of water mites from the genus *Arrenurus* use a variety of adult insects as parasitic hosts, and these insects provide the primary means of dispersal. This study examines how the loss of parasitism in three *Arrenurus* lineages has impacted population differentiation. In addition, the relationship between host use and patterns of genetic variation has been investigated in this genus. As a model system, water mites provide a unique opportunity to test the degree to which population differentiation actually reflects the ongoing dispersal of individuals in a landscape far from a drift–gene flow equilibrium.

Materials and methods

Life history of Arrenurus *water mites*

Nearly all species of water mite larvae (Acari: Hydrachnida) are obligate ectoparasites on aquatic insects (Smith & Cook, 1991). After hatching from eggs, mite larvae seek out insect larvae and pupae. As the insects emerge, the mites attach to the winged adult and begin to feed. When their hosts later return to water to mate or oviposit, the engorged mites release and fall to the pond, lake or stream bottom. In most genera, the adult portion of the life cycle is then spent in a free-living state. Reproduction is sexual. Thus, the mites disperse parasitically only once in their lives, although dispersal presumably occurs by other unknown means on rare occasions. Water mites in the genus *Arrenurus* parasitize nematoceran flies (e.g. tanypodine midges, chaoborids and mosquitoes) or odonates (libellulid dragonflies and damselflies), and individual species of mites are faithful to only one of these insect taxa nearly 100% of the time (Stechmann, 1978).

In contrast to the generalized life cycle, three species of *Arrenurus* have independently lost the ability to parasitize insects (two in subgenus *Truncaturus* and one in subgenus *Megaluracarus*). Eggs of these species hatch directly into a postlarval stage that possesses no means of parasitic dispersal, analogous to the direct-developing life history found in some marine invertebrates. This life cycle has evolved independently over 20 times in at least 14 genera (Smith, 1998).

Collection of mites

From 1994 to 1996, water mites were collected using standard techniques (see Smith & Cook, 1991) from sites throughout New York State and Ontario, Canada (Fig. 1). An attempt was made to sample as many individuals in the genus *Arrenurus* as possible from a diversity of bogs, ponds, seepage areas, lakes and slow rivers. Although most of the species were sampled in habitats small enough to support only a single population, it seemed probable that separate bays of large lakes and rivers contain multiple populations of the same species. In order to minimize sampling artifacts imposed by habitat size, collections in large lakes and rivers were restricted to areas of ≈ 200 m^2 or less.

Sample sizes for nine species and two species complexes were large enough for analyses of heterozygosity and population differentiation (see below). Within these taxa, geographical distances between pairs of populations ranged between 300 m and 385 km, and the mean pairwise distance ranged between 56 km (for *A. rufopyriformis*) and 189 km (for *A. danbyensis*).

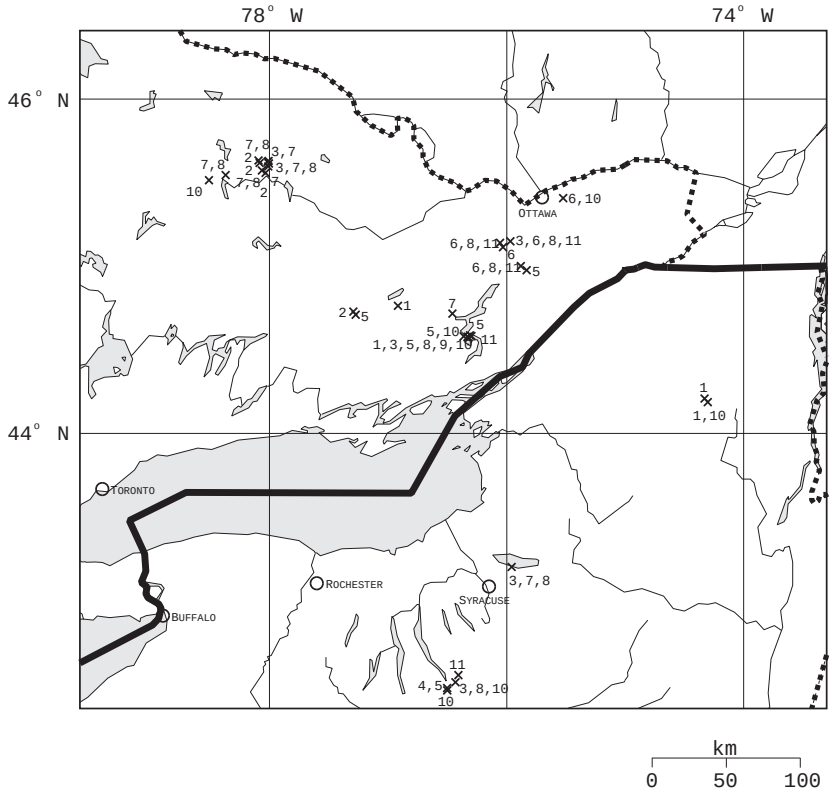

Fig. 1 Collection sites. Taxa are numbered as in Table 1.

For most of the *Arrenurus* species in this study, sampling occurred in populations near the limits of a broad geographical range (Crowell, 1961; Mullen, 1974; B. Smith and I. Smith, pers. comm.). *Arrenurus manubriator*, *A. birgei*, *A. danbyensis* and *A. planus* are found throughout most of eastern North America, ranging from Ontario to the Gulf coast. The distributions of *A. angustilimbatus* and the *A. mamillanus*/*neomamillanus* and *A. kenki*/*palustris* species complexes overlap with these four species in Ontario and New York and stretch north into Manitoba and Quebec. *Arrenurus apetiolatus* is found throughout both North America and South America. The presumed midge parasite *A. n.* sp., nr. *manubriator* has been found in sites in the north-eastern United States, eastern Canada, North Carolina and the south-central United States and seems to possess a distribution that circumscribes its directly developing sister species *A. manubriator* with little geographical overlap (B. Smith, pers. comm.). Similarly, populations of the direct developer *A. rufopyriformis* are restricted to three centres that are surrounded by populations of *A. angustilimbatus* (five known populations near Ottawa, Ontario, a single population in New Jersey and several populations in Michigan). For logistical reasons and because of the possibility that *A. rufopyriformis* in these three areas do not represent a monophyletic taxon, the *A. rufopyriformis* populations outside of Ontario were not included in the study. Only a single population of the direct developer *A. n.* sp., nr. *danbyensis* is known.

Electrophoresis

Individual adult mites were identified with published keys (Cook, 1954a,b, 1955; Mullen, 1976), verified by B. Smith (Ithaca College, NY, USA) and stored live at 8°C for periods of time that ranged from several weeks to more than a year. To promote metabolic activity, mites were warmed to room temperature and fed ostracods (their natural food) for 2 days before electrophoresis. Individuals kept in the laboratory for extended periods of time produced electrophoretic patterns that did not appear to differ from recently collected individuals.

Nine isozymes representing 15 putative loci were screened using a cellulose acetate system (protocols and stains modified from Hebert & Beaton, 1993). Five isozymes were run in a Tris/glycine (TG) buffer: ADH (EC 1.1.1.1), ARK (2.7.3.3), FUM, two loci (4.2.1.2), GPI, two loci (5.3.1.9) and PGM, two loci (5.4.2.2). Four isozymes were run in a citric acid/aminopropylmorpholine (CAAPM) buffer system: AAT, three loci (EC 2.6.1.1), G3PDH (1.2.1.12), MDH, two loci (1.1.1.37) and 6PGDH (1.1.1.44). Individual mites were

typically large enough to permit staining for 12–15 gels. In all cases, heterozygotes stained according to known quaternary structure. Marker alleles consisting of *Daphnia* from a clonal laboratory line were run in two lanes on every gel.

Twenty-six taxa were collected from 50 sites. From this data set, over 1100 individuals from 11 taxa in which 10 or more individuals had been sampled were analysed. These taxa included two species complexes for which taxonomy is unreliable and in which a number of cryptic species are thought to be present (*A. kenki*/*palustris* spp. and *A. mamillanus*/*neomamillanus* spp.). Two currently undescribed species were also included in the study (*A. n.* sp., nr. *manubriator* and *A. n.* sp., nr. *danbyensis*). Although no published phylogeny is currently available for the genus *Arrenurus*, traditional subgeneric affiliations and recently recognized sister species are presented in Table 1.

Estimation of heterozygosity, population differentiation and gene flow

Observed (direct count) heterozygosities averaged across individuals and loci (H_o) were calculated in each of the 11 taxa. Because of unequal sample sizes among populations, heterozygosity was also calculated by averaging across (sub)populations (H_o[s]).

Population differentiation was estimated using *F*-statistics in seven species that were collected from more than one population. (Because species could not be separated reliably in the *A. mamillanus*/*neomamillanus* and *A. kenki*/*palustris* species complexes, population differentiation was not quantified in these taxa.) Mean squares for the calculation of f_{IS}, F_{IT} and Weir and Cockerham's θ (an estimator of F_{ST}) were obtained by using indicator variables for each allele and generating nested analysis of variance tables for each allele at each locus (Weir, 1990). Variance estimates for the *F*-statistics were determined by jackknifing across alleles and from 500 bootstraps conducted across loci for each species (Weir, 1990).

The relationship between θ and log (geographical distance) was examined for all pairwise combinations of populations in the seven species for which an overall θ was calculated (Slatkin, 1994). No isolation by distance was detected over the scale for which populations were sampled. Accordingly, an island model estimate of F_{ST} was assumed to be an appropriate descriptor of population differentiation, and gene flow was estimated as Nm, the number of migrants per generation, using the relationship $Nm = [(1/\theta) - 1]/4$.

Correlations with loss of parasitism

Host type (or the absence of parasitism) is expected to remain constant over the evolutionary lifetime of each mite species, because it is phylogenetically conservative within the genus *Arrenurus* and across other mite genera (see above). Thus, if host use can be reliably and categorically ranked from low to high dispersal ability, it should provide an accurate, qualitative metric of dispersal. If the ranking is truly categorical, it is a long-term estimate not subject to extreme fluctuations in any particular generation. Because of this, clear life history differences related to dispersal ability often correlate well with long-term averages of population differentiation such as F_{ST} (Bohonak, 1999).

Table 1 Subgeneric affiliations of *Arrenurus* water mites in this study (Cook, 1954a,b, 1955; Mullen, 1976), number of individuals and number of populations surveyed

Subgenus	Species	Insect host	*n*	Number of populations
Megaluracarus	1. *A. manubriator*	Direct developer	44	4
	2. *A. n.* sp., nr. *manubriator*	Midge	93	3
	3. *A. mamillanus*/*neomamillanus* spp. complex	Midge	14	4
	4. *A. apetiolatus*	Midge	27	1
	5. *A. birgei*	Midge	70	5
Truncaturus	6. *A. rufopyriformis*	Direct developer	336	5
	7. *A. angustilimbatus*	Mosquito	265	7
	8. *A. kenki*/*palustris* spp. complex	Mosquito	62	10
	9. *A. n.* sp., nr. *danbyensis*	Direct developer	18	1
	10. *A. danbyensis*	Mosquito	143	8
Arrenurus	11. *A. planus*	Odonate	26	4

Hypothesized sister species (1–2, 6–7 and 9–10) are bracketed by double lines (Smith, 1998; unpubl. data). Direct developers are those species that have lost the ability to parasitize insects. *Arrenurus rufopyriformis* is synonymous with *A. lacrimatus* Cook (see Mullen, 1974, 1976).

It was assumed that nonparasitic species disperse less than their parasitic sister species. Water mite adults are obligately aquatic, and species that forgo parasitism can only disperse on rare occasions arising from chance events (e.g. wind, birds). If habitats such as lakes and rivers are large enough to include multiple populations of the same species, it is possible that adults may also disperse by swimming. However, both parasitic and nonparasitic adults should disperse equally well via birds, mammals or swimming.

It was assumed that population differentiation would be greater in species that have lost the ability to parasitize insects. This was tested by comparing θ between the sister species *A. manubriator* and *A. n.* sp., nr. *manubriator*, and between the sister species *A. rufopyriformis* and *A. angustilimbatus*. Statistical significance was tested by comparing 95% confidence intervals obtained by jackknifing and by bootstrapping over loci.

Heterozygosity is also expected to depend on dispersal ability, with parasitic species possessing higher observed heterozygosity. This is because gene flow between populations should increase effective population size and slow the rate of drift. (In the absence of gene flow and new mutations, populations will eventually lose all variation; conversely, effective population size will be maximal when gene flow is so high as to preclude population subdivision.) Three sister species pairs can be contrasted for heterozygosity (Table 1). Because electrophoretic variation is higher for some loci across all species (see Table 2), statistical significance for the contrasts was determined by comparing species on a locus-by-locus basis using a one-sided, two-sample sign test.

Correlations with host type

Variation in host type permits a more generalized hypothesis relating dispersal ability to population genetic metrics. Parasitic species of water mites tend to be highly specific to particular insect taxa; however, only one mite studied here (*A. danbyensis*, subgenus *Truncaturus*) is restricted to a single host species of insect (the mosquito *Coquilletidia perturbans*). Other *Truncaturus* mites are more opportunistic, parasitizing multiple mosquito species as available. Similarly, the remainder of the mites are found on many insect species within the same higher taxon (Smith & Oliver, 1986; Smith & Cook, 1991). Phylogenetic patterns of host use are relatively conservative, with nearly all mosquito parasites restricted to the subgenus *Truncaturus*, midge parasitism found primarily in the subgenera *Megaluracarus* and *Micruacarus* and odonate parasitism found only in the subgenus *Arrenurus*. As a result of this host specificity, an attempt was made to rank dispersal ability in each mite species based on the subfamily, family or order of its insect host. [Because dispersal in *C. perturbans* seems to be typical of mosquitoes in northeastern North America (e.g. Johnson, 1969), no distinction in dispersal ability was made between *A. danbyensis* and the other mosquito parasites.]

A review was made of the available literature on dispersal in the taxa parasitized by *Arrenurus* mites in

Table 2 Observed heterozygosity averaged by individual (H_o) for each locus with more than one allele

	Species										
	None*			Tanypodine midge*				Mosquito*			Odonate*
Locus	1	6	9	2	3	4	5	7	8	10	11
AAT-1	0.02	0.04	0.00	0.00	0.38	–	0.00	0.07	0.04	0.03	0.00
AAT-2	0.00	0.12	0.00	0.10	0.00	–	–	0.29	0.17	0.12	–
AAT-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27
ADH	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.16	0.03	0.00
ARK	0.18	0.01	0.00	0.00	0.00	0.00	0.03	0.04	0.03	0.02	0.04
FUM-2	0.32	0.54	0.00	0.13	0.14	0.07	0.19	0.56	0.24	0.02	0.08
GPI-1	0.00	0.02	0.00	0.00	0.43	0.00	0.10	0.09	0.25	0.50	0.15
MDH-1	0.00	0.04	0.00	0.00	0.00	0.05	0.05	0.01	0.00	0.03	0.11
PGM-1	0.50	0.52	0.00	0.38	0.43	0.30	0.52	0.58	0.43	0.33	0.44
Overall H_o	0.073	0.092	0.000	0.043	0.099	0.035	0.071	0.118	0.093	0.078	0.084
Overall H_o[s]	0.078	0.093	0.000	0.039	0.111	0.035	0.074	0.123	0.113	0.064	0.083

*Dispersal vector.
Averages over loci are presented for H_o and for observed heterozygosity averaged at subpopulation level (H_o[s]). Species numbers are the same as in Table 1.

North America. Of these taxa, odonates (damselflies and libellulid dragonflies) seemed to possess the most variability, with continental migrations being reported for some species and high degrees of territoriality in others. However, even in odonates that possess territorial males, males forage away from their territory, and both sexes disperse during the teneral phase before territory acquisition (Corbet, 1980). For example, Cook (1991) recaptured only 22% of marked teneral males in the territorial *Leucorrhinia frigida* (an *Arrenurus* host), in contrast to 61% of mature males (0% of teneral and 0.6% of adult females were recaptured). Overall, odonates were judged as likely to disperse mites more often and further than the other insect taxa, with single odonates often carrying hundreds of mites and dispersing over tens or hundreds of kilometres (e.g. Mitchell, 1959, 1962, 1965; Johnson, 1969; Cook, 1991; Miyakawa, 1994). Dispersal in mosquitoes has been studied more extensively. In contrast to odonates, the mosquito species used by *Arrenurus* are less heavily parasitized (typically 1–11 mites) and tend to disperse 10 km or less (e.g. Johnson, 1969; Jalil & Mitchell, 1972; Mullen, 1974; Brust, 1980; Service, 1993; Jensen & Washino, 1994). The dispersal literature on midges is exceedingly small; nonetheless, it was hypothesized that tanypodine midges disperse fewer mites for shorter distances than either mosquitoes or odonates. Dispersal distances for tanypodine midges are typically < 100 m from the site of emergence, and less than five mites are usually found per host (e.g. Efford, 1963; Booth & Learner, 1978; Lanciani, 1978; LeSage & Harrison, 1980; Kouwets & Davids, 1984; Jackson & Resh, 1989). Thus, nonparasitic species, midge parasites, mosquito parasites and odonate parasites were assigned dispersal ranks of 1, 2, 3 and 4, respectively. Although numerous other ecological and demographic differences between the mites undoubtedly exist, the restriction of this study to a single genus provides some assurance that the species studied were as biologically similar as possible.

Spearman rank correlations of the dispersal ranking with F_{ST}, H_o and H_o[s] were used to assess whether dispersal was related to genetic variation at the level of the genus. Significance values for one-tailed tests were obtained from Daniel (1987).

Results

Of the 15 loci scored, six were monomorphic within all species (*FUM-1, GPI-2, G3PDH, MDH-2, PGM-2* and *6PGDH*). The remaining nine were assayed in all taxa with the exception of *AAT-1* and *AAT-2*, for which staining was problematic in smaller individuals. Allozyme variation in *Arrenurus* was very low, with average heterozygosities across 15 loci ranging from 0.12 in *A. angustilimbatus* to 0.00 in *A. n.* sp., nr. *danbyensis* (Table 2). A high degree of variation existed among loci and, although *PGM-1* and *FUM-2* tended to display the highest heterozygosities, no other patterns were apparent.

Tests for departure from Hardy–Weinberg equilibrium were conducted on all locus–population combinations for which more than one allele was present, excluding the two complexes of cryptic species. Seventeen out of 138 tests were significant at $\alpha = 0.05$. (After Bonferroni correction for multiple tests, only 10 departed significantly from Hardy–Weinberg.) In many of these cases, rare alleles and small to moderate sample sizes led to expected genotype frequencies less than 1. When rare alleles were pooled, seven tests remained significant. Estimates of the inbreeding coefficient f_{IS} were low in all taxa, with the exception of *A. birgei*, in which $f_{IS} = 0.16$ (Table 3). The high f_{IS} in *A. birgei* was caused by the presence of one individual in each of two populations that was homozygous for alleles that were rare in that population. The loci involved were *ADH* and *GPI-1* in each case. Because the mites were collected at a time of year after dispersal but before breeding, these individuals were likely to be immigrants. When these animals were eliminated from the analysis, f_{IS} dropped to 0.02, but population differentiation remained high ($\theta = 0.20$).

Population differentiation was almost nonexistent in five of the seven species for which *F*-statistics were calculated (Table 3). However, four species possessed θ values significantly greater than zero (based on jackknifed 95% confidence intervals). Estimates of θ were slightly negative in *A. n.* sp., nr. *manubriator*, but the departure from zero was small and nonsignificant. Population differentiation in *A. birgei* and *A. planus* was large in comparison with the five remaining species, although variances were also highest in these two species. Assuming equilibrium and an island model of dispersal, gene flow estimates were high in all species ($Nm = 0.6$ or more individuals per generation; Table 3).

Correlations with loss of parasitism

Two comparisons of population differentiation between sister species possessing and lacking parasitism were possible. For each of these comparisons (*A. n.* sp., nr. *manubriator* vs. *A. manubriator* and *A. angustilimbatus* vs. *A. rufopyriformis*), the species lacking a means of larval dispersal possessed higher estimates of θ and lower estimated gene flow than species that parasitize insects (Table 4). Estimates of θ in *A. angustilimbatus* were approximately one-third of those in *A. rufopyriformis* and significantly lower according to jackknifed

Table 3 Population differentiation in seven *Arrenurus* species calculated according to the method of Weir (1990)

	Species										
	1 *A. manubriator*	6 *A. rufopyriformis*	5 *A. birgei*	*A. n.* sp., nr. *manubriator*	2	*A. angustilimbatus*	7	*A. danbyensis*	10	*A. planus*	11
Dispersal vector	None	None	Midge	Midge		Mosquito		Mosquito		Odonate	
Estimate											
F_{IT}	0.0095	0.116	0.30	0.098		0.072		−0.029		0.30	
f_{IS}	0.0034	0.086	0.16	0.103		0.061		−0.034		0.03	
θ (jackknife SE)	0.0061 (0.0220)	0.032 (0.001)	0.16 (0.07)	−0.005 (0.006)		0.012 (0.003)		0.0042 (0.0038)		0.28 (0.06)	
95% CI for θ (jackknife across loci)	−0.055 to 0.031	0.031 to 0.034	0.03 to 0.29	−0.020 to 0.004		0.007 to 0.020		−0.003 to 0.012		0.20 to 0.44	
95% CI for θ (bootstrap over loci)	−0.031 to 0.189	0.018 to 0.038	−0.03 to 0.42	−0.033 to 0.017		0.001 to 0.023		−0.021 to 0.017		−0.05 to 0.42	
Nm estimate	40.9	7.6	1.3	[∞]		19.9		58.8		0.6	

Estimates of error were calculated by jackknifing across loci, and from the central 95% of 500 bootstraps conducted across loci.

estimates of variance ($P < 0.05$). (For variance estimates bootstrapped across loci, the contrast was not significant; $0.05 < P < 0.10$.) The differences in population differentiation between *A. manubriator* and *A. n.* sp., nr. *manubriator* were not significant ($P > 0.10$ for both jackknifed and bootstrapped estimates of variance).

In two of the three sister species contrasts, direct-developing species possessed significantly less allozyme heterozygosity than their parasitic counterparts ($P = 0.04$, $P = 0.004$; Table 4). However, *A. manubriator* possessed more heterozygosity than *A. n.* sp., nr. *manubriator*, contrary to expectations.

Correlations with host type

There was some indication that heterozygosity increased across the four dispersal categories, with widely dispersing species tending to possess higher average heterozygosities than poorly dispersing species (Fig. 2). However, a high degree of variation existed within each dispersal category, and the odonate parasite thought to possess the highest dispersal ability (*A. planus*) displayed only moderate levels of heterozygosity. The rank correlation of H_o with dispersal ability was not significant ($r_s = 0.45$; $0.05 < P < 0.10$; r_s for H_o[s] $= 0.38$, $P > 0.10$). There was also no relationship between population differentiation and dispersal ability ($r_s = -0.24$, $P > 0.10$; Fig. 3).

Discussion

Loss of parasitism in *Arrenurus* was only weakly associated with population genetic metrics in this study. Contrasts between sister species showed a slight increase in θ when parasitic dispersal was not possible, but interpretation of the results is complicated by near panmixia in five of the seven species for which θ was measured. In two out of three cases, the loss of parasitism was also associated with significant reductions in heterozygosity.

Relationships between allozyme variation and inferred dispersal ability were not apparent at the level of the genus. Across 11 species and species complexes of *Arrenurus* water mites, heterozygosity was not significantly correlated with dispersal tendency, despite the fixation of all 15 loci in one of the nondispersing species (*A. n.* sp., nr. *danbyensis*). Independent contrasts between *Arrenurus* taxa at higher phylogenetic levels (e.g. subgenera) would be more appropriate than using all 11 species as independent data points; however, many relationships among *Arrenurus* species are unknown at this time. Isolation by distance was not detected in seven species collected from multiple popu-

Table 4 Comparison of H_o and θ between sister species with parasitic and direct-developing life cycles

Contrast		Heterozygosity	
Parasitic species–direct developer	Population differentiation	Direction	Sign test
A. n. sp., nr. *manubriator* (2)–*A. manubriator* (1)	**2 < 1**	2 < 1	$P = 0.97$
A. angustilimbatus (7)–*A. rufopyriformis* (6)	**7 < 6***	**7 > 6**	$P = 0.04$
A. danbyensis (10)–*A. n.* sp., nr. *danbyensis* (9)		**10 > 9**	$P = 0.004$

Contrasts in bold are consistent with *a priori* expectations.
*θ is significantly ($\alpha = 0.05$) higher in *A. angustilimbatus* than in *A. rufopyriformis* when variance is calculated by jackknifing over loci, but not when bootstrapping (see Table 3).

lations (see Materials and methods), which may suggest strong founder effects or that long-distance dispersal is common. Furthermore, populations of five species were essentially panmictic across geographical scales up to several hundred kilometres, with θ values of 0.032 or less.

Boileau *et al.* (1992) studied allozyme population structure in Canadian populations of freshwater invertebrates, although water mites were not included in their survey. They concluded that dispersal is not related to population differentiation in this region of North America by comparing 15 species of Turbellaria, Anostraca, Cladocera, Copepoda, Ostracoda and Collembola. Despite a more restricted taxonomic focus in this study, the conclusions are generally similar, with one notable exception. The average estimate of F_{ST} in the taxa studied by Boileau *et al.* (1992) was 0.12, and all values were significantly greater than zero. Boileau *et al.* (1992) interpreted the lack of fit between gene flow estimates and dispersal (as inferred from morphological and behavioural characters) as being the result of pronounced founder effects, which led to an initial state of high population differentiation. In contrast, the water mites studied here more often possess estimates of F_{ST} that seem extraordinarily low.

Why would extreme departures from equilibrium lead to such different results? One possibility is that species from each study differ in the ways that they colonize new populations. For most of the taxa considered by Boileau *et al.* (1992), dispersal is infrequent and temporally variable, and populations are likely to be founded by one or only a few individuals. Population sizes can quickly reach 10^5–10^8 individuals, particularly in asexual or parthenogenetic freshwater invertebrates such as the crustacean *Daphnia*. These conditions are likely to

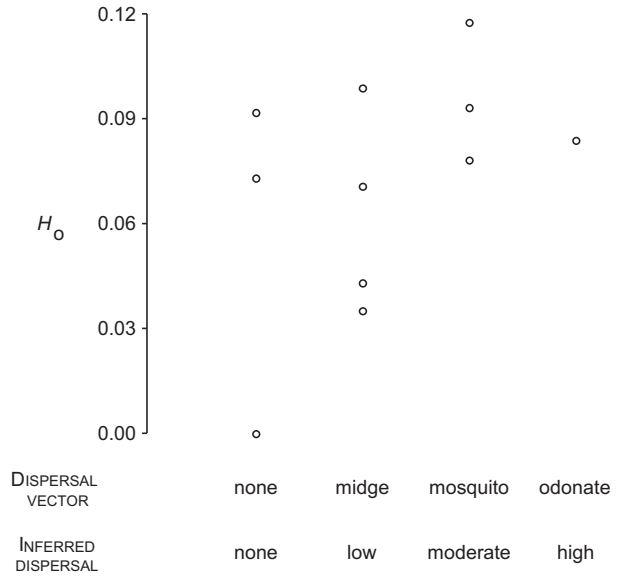

Fig. 2 Average observed heterozygosity as a function of dispersal vector in *Arrenurus*.

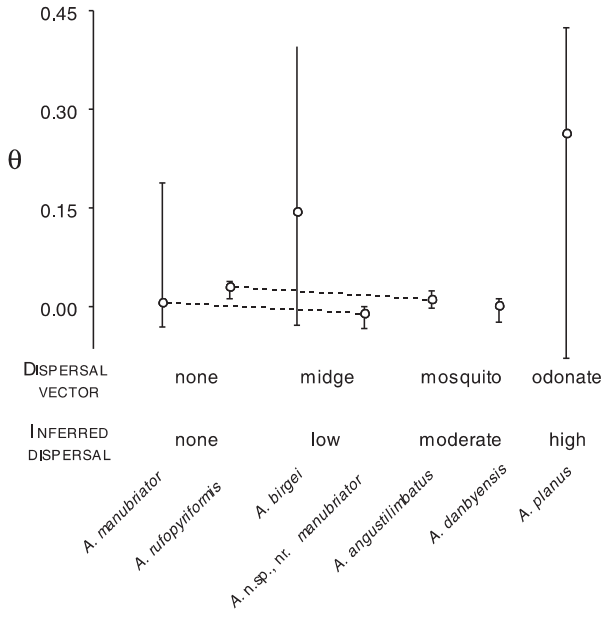

Fig. 3 Estimates of population differentiation (θ) as a function of dispersal vector. Dashed lines connect closely related sister species (see Table 1). Error bars are 95% confidence intervals from bootstraps conducted across loci.

promote marked founder effects and create high initial values of F_{ST}.

In contrast, water mites are obligately sexual and disperse as juveniles rather than propagules resistant to drying. Dispersal via insect hosts may also be less variable from generation to generation than the unknown vectors that move most freshwater invertebrates. More importantly, the frequent existence of multiple mite larvae on each dispersing insect (e.g. Efford, 1963; Mitchell, 1965) means that new populations of many species would be colonized by multiple individuals. The size of founding populations could also be enhanced by the simultaneous dispersal of many winged insects by meteorological events (e.g. Mitchell, 1962). Thus, range expansions of mosquitoes, midges and odonates from glacial refugia at the end of the Pleistocene would be more likely to lead to genetically homogeneous populations of water mites with F_{ST} values close to zero than to high initial population differentiation. Because of this, separating the population genetic consequences of dispersal ability and population history in this system will require more rapidly evolving markers than allozymes. Even when sister species with divergent life histories can be collected in large numbers (such as *A. rufopyriformis* and *A. angustilimbatus*), it is statistically difficult to test the comparative hypothesis that F_{ST} estimates of 0.01 and 0.03 are different.

The only other study to date on allozyme differentiation in water mites provides support for the hypothesis that most of the *Arrenurus* species in this study are not in equilibrium. Edwards & Dimock (1997) estimated F_{ST} as 0.12 for four populations of the midge parasite *Unionicola formosa* in North Carolina and 0.31 for six populations of *U. foili* throughout the south-eastern United States. Because freshwater habitats in North Carolina are likely to be older than those in New York and Ontario, it is notable that population differentiation is higher for both *Unionicola* species than for the midge parasite *A. n.* sp., nr. *manubriator* in this study. However, both the geographical scale of sampling and the F_{ST} estimate of 0.16 for the midge parasite *A. birgei* are intermediate between the two *Unionicola* species. These data are consistent with the hypothesis that the two *Arrenurus* species with relatively high amounts of population differentiation (*A. birgei* and *A. planus*) are closer to a drift–gene flow equilibrium than those with very low amounts of differentiation. A study of latitudinal patterns of differentiation in widespread species would help to corroborate or refute this hypothesis.

Several alternative interpretations are also possible. The *Arrenurus* species might not be ecologically and demographically comparable with *U. formosa* or *U. foili* and may be much closer to equilibrium than the invertebrates studied by Boileau *et al.* (1992). The populations in this study are further south than those sampled by Boileau *et al.* (1992) and, as a result, the mites may have had considerably more time to reach an equilibrium between drift and gene flow. The ponds of Boileau *et al.* (1992) are thought to have been created by glacial rebound approximately 3000 years ago. In contrast, glacial influences in south-central Ontario lasted until the end of the Wisconsin glaciation (14 000–10 000 years BP), and invasions of most freshwater fish from refugia occurred between 12 000 and 9000 years BP (Mandrak & Crossman, 1992). Although some of the lakes, bogs and ponds from this region are likely to have been created by more recent geological and human activities, many are considerably older than 3000 years. In this case, near-zero values of θ would reflect high gene flow between bodies of water that are closely spaced relative to dispersal potential.

However, several lines of evidence suggest that patterns of allozyme variation are primarily a result of historical factors. First, isolation by distance was not evident in any species over geographical scales ranging between hundreds of metres and hundreds of kilometres. Secondly, the poor fit of host type with gene flow is inconsistent with an equilibrium interpretation, unless host type is not a good indicator of dispersal ability. An analysis of population structure that uses a larger geographical scale and additional species will be necessary for more detailed inferences. In addition, further ecological and genetic studies will be needed to reconcile gene flow estimates of 41 and seven individuals per generation in the nonparasitic, direct-developing species *A. manubriator* and *A. rufopyriformis* with the assumption that these mites possess no means of active dispersal.

Before approaching an equilibrium between drift and gene flow, gene frequencies can assume patchy distributions, clinal patterns of variation or more complicated mosaics (e.g. Nürnberger & Harrison, 1995). For these reasons, the interplay between gene flow, random drift, history and natural selection on one (or many) loci usually leads to a number of equally plausible interpretations for any individual species. Studies of phylogenetically and ecologically comparable groups of species can be useful in discriminating among these alternatives. Although variation in the relatedness among organisms is typically regarded as a confounding factor in evolutionary studies, exploiting these relationships is one way to determine the relative impacts of history and contemporary gene flow on population structure. The results of this study verify that, in regions where historical effects are pronounced, a synthetic approach is most informative when focused at the lowest taxonomic level. Studies on closely related, yet ecologically

divergent, species will provide the greatest insight for these types of systems.

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