Freshwater Biology (2004) **49**, 170–180

Distributional, morphological and genetic consequences of dispersal for temporary pond water mites

ANDREW J. BOHONAK,* BRUCE P. SMITH† AND MELISSA THORNTON†
*Department of Biology, San Diego State University, San Diego, CA, U.S.A.
†Department of Biology, Ithaca College, Ithaca, NY, U.S.A.

SUMMARY

1. To determine the consequences of dispersal and gene flow for temporary pond water mites (Hydrachnida), we compared distributional, genetic and morphological characters in the closely related species *Arrenurus angustilimbatus* and *A. rufopyriformis*. The former has larvae that parasitise and disperse on adult mosquitoes, whereas larvae of the latter forego any association with hosts.
2. Allometrically adjusted egg size and gonopore size were found to be useful characters for distinguishing between females of the two species.
3. *Arrenurus angustilimbatus* possesses a broader and more continuous geographic distribution than its 'direct developing' counterpart. Allozyme heterozygosity was higher and population differentiation lower in *A. angustilimbatus*. In addition, populations of *A. rufopyriformis* were morphologically divergent, whereas populations of *A. angustilimbatus* were not. Isolation by distance analyses on both genetic and morphological characters indicated that the results were not biased by different sampling regimes for the two species.
4. These results demonstrate the importance of mosquito parasitism for maintaining ecological and genetic linkages between *A. angustilimbatus* populations. More broadly, we hypothesise that insect-mediated dispersal has contributed to the ecological and evolutionary success of water mites, because the Hydrachnida lack other obvious adaptations for dispersing in space or time.

Keywords: *Arrenurus*, body size, geographic distribution, gonopore size, population genetic structure

Introduction

Organisms that live in temporary ponds face numerous physiological and ecological challenges. Factors such as salinity and predation pressure tend to change dramatically as a pond dries, and unpredictable rainfall patterns often dictate stochastic variation in pond duration (e.g. Skelly, 1996; Simovich & Hathaway, 1997). Numerous authors have suggested that in the absence of extreme phenotypic plasticity, there are only three general strategies that might allow populations of freshwater invertebrates to persist despite environmental variability. These include the ability to disperse spatially to new populations, the ability to disperse temporally through extended propagule dormancy, and an extended adult life span (Hairston, 1996; Hairston & Cáceres, 1996; Williams, 1998; Brock *et al*., 2003).

Within this framework, the great species diversity possessed by the water mites (Acari: Hydrachnida) in freshwater habitats is remarkable. Without the benefits of an extended diapause, a long-lived adult stage, or dispersal via terrestrial adults, water mites have radiated since the Triassic or earlier to over 5000 described species, occupying nearly all available freshwater habitats (Viets, 1987; Smith, Cook & Smith, 2001). Conceivably, their ecological and evolutionary successes may be attributed to the use of insects as

Correspondence: Andrew J. Bohonak, Department of Biology, San Diego State University, San Diego, CA 92182-4614, U.S.A.
E-mail: bohonak@sciences.sdsu.edu

dispersal vectors. After hatching from eggs, larvae of most Hydrachnida (including the three largest superfamilies) seek out insect larvae or pupae, and attach to the winged terrestrial adults as they emerge (Smith & Oliver, 1986; Smith, 1988). The larvae then feed parasitically as their hosts leave the natal habitat and fly away (as is the case with most insects). Parasitism is obligate, because mites that fail to find an appropriate host die. In many taxa, engorged mite larvae release from the hosts when they oviposit or mate at the water's surface, and then complete their life cycle as aquatic, predatory adults. Each mite species tends to associate with insects at taxonomic levels higher than species (e.g. order, family, or several closely related families; reviewed by Smith & Oliver, 1986; Smith, 1988). For example, mites in the genus *Wandesia* parasitise only particular families of stoneflies.

Obligate larval parasitism is the typical, and presumably plesiotypic (ancestral) condition in parasitengone mites, which includes the water mites (Smith, 1998). However, a number of 'direct developing' species have been found within disparate taxonomic groups (reviewed by Smith, 1998). In a life-history strategy analogous to 'direct development' in marine invertebrates, larvae of these species hatch from unusually large eggs, forego a parasitic larval stage, and do not associate with hosts. Approximately 5% of the species for which life-history patterns have been confirmed do not feed parasitically; the interpretation is that loss of parasitism in water mites is a recent and recurring event in evolutionary history, and ultimately a short-lived (dead-end) phenomenon (Smith, 1998). Presumably, forgoing parasitism avoids inherent risks to survivorship and the need to synchronise with a host's life cycle, and facilitates more generations per year. However, the loss of host-mediated dispersal is likely to decrease the ability of populations and species to persist in spatially variable environments.

In the genus *Arrenurus*, four species are known to be direct developers, and each of the four closely resembles a species with parasitic larvae. In North America, adults of *Arrenurus* new species, near *manubriator* closely resemble adults of *Arrenurus manubriator* (sensu stricto, Marshall, 1903). However, the latter species lacks larval host association. Similarly, *A. danbyensis* Mullen (1976) is morphologically similar to *A.* n. sp., nr. *danbyensis* (a direct developer). A third example is found in *A. angustilimbatus* and its presumed sister species *A. rufopyriformis* (a direct developer). Finally, a pair of undescribed species or divergent populations (close to *A. forpicatoides* Lundblad (1941)) has been found in Australia, with one species of the pair forgoing larval parasitism. The four species with non-parasitic larvae are clearly from divergent evolutionary lineages, because no two are similar enough morphologically to suspect that they are closely related. *Arrenurus rufopyriformis* and the new species near *A. danbyensis* belong to different subgroups within the subgenus *Truncaturus*; *A. manubriator* is in another subgenus; and the unnamed species near *A. forpicatoides* no. 2 is in yet a different subgenus (Cook, 1954, 1955, 1974; Mullen, 1976). In addition, the presence or absence of parasitic larvae corresponds precisely to disjunct distributions in both larval and adult morphological traits. Extensive sampling throughout northeastern North America (e.g. Smith & McIver, 1984b; Smith, 1990a, 1998; Bohonak, 1999) has shown that direct developers and their parasitic counterparts coexist regionally but never in the same pond.

We focused our study on the species pair *A. angustilimbatus* and *A. rufopyriformis*. Because of morphological similarity, their taxonomic status has only recently been resolved. Adults described as *A. rufopyriformis* Habeeb (1954) and *A. lacrimatus* Cook (1955) are cryptically similar to *A. angustilimbatus* Mullen (1976). In fact, *A. angustilimbatus* was named solely from the larval stage (which parasitises mosquitoes), and both *A. rufopyriformis* and *A. lacrimatus* were named from adult males. These problems with nomenclature were resolved by examining larvae hatched from eggs laid by females in the laboratory, and rearing field-collected engorged larvae to the adult stage (Smith & McIver, 1984a). In addition to the absence of parasitic associations, *A. rufopyriformis* larvae are morphologically distinctive, with relatively wide body sclerites, reduced leg length, reduced setal length and reduced sclerotisation in comparison with *A. angustilimbatus*. Adult *A. rufopyriformis* females have a relatively larger gonopore (genital opening), through which larger eggs are extruded (Cook, Smith & Brooks, 1989). Laboratory experiments have also shown that each life-history morph within the species pair breeds true (B.P. Smith, unpublished data). Studies of these taxa throughout Ontario, Canada, and from the type locality of *A. rufopyriformis* lead us

to conclude that *A. rufopyriformis*, *A. lacrimatus* and the direct-developing Ottawa populations collectively represent one morphospecies. This species is designated *A. rufopyriformis* because of precedence over the name *A. lacrimatus*.

If dispersal ability is a key life-history trait for water mites, differences in host use should influence distribution, population structure, speciation and extinction. We used contrasts between *A. angustilimbatus* and *A. rufopyriformis* to infer the consequences of dispersal and gene flow via adult mosquitoes for patterns of distribution, genetic diversity and morphological diversity. The genetic analysis of Bohonak (1999) was extended, and supplemented with distributional and morphological data. Our results demonstrate the utility of *Arrenurus* as a model system for studying the consequences of insect-mediated dispersal for water mites, and for developing a broader understanding of life-history evolution in freshwater invertebrates.

Methods

Conceptual background

To ascertain the consequences of dispersal and gene flow, we contrasted species distributions, genetic variation and morphology of *A. rufopyriformis* with *A. angustilimbatus*. Three important assumptions underlie these contrasts. First, *A. angustilimbatus* and *A. rufopyriformis* are assumed to represent reproductively isolated lineages (species). This conclusion (based on the experiments described above) is further supported by the distribution of populations sampled for this study from Ontario, Canada. All populations of *A. rufopyriformis* are found geographically on the same glacial rebound plateau, and no *A. angustilimbatus* populations exist between any two *A. rufopyriformis* populations. No hybrids are found in nature, although they have been produced in some laboratory crosses (B.P. Smith, unpubl. data).

Secondly, these taxa are assumed to represent sister species, so that statistical contrasts between them can be justified in an evolutionary framework (Harvey & Pagel, 1991). Finally, we interpret our results by assuming that *A. rufopyriformis* disperses less often than *A. angustilimbatus* because larvae on the latter are mosquito ectoparasites. The temporary pools in which these species occur are discrete, spatially separated waterbodies without interconnecting streams or rivers. Therefore, dispersal in the adult stage is highly unlikely except for rare chance events (e.g. by wind, birds, mammals; Maguire, 1963; Peck, 1975; see Bohonak & Jenkins, 2003).

Species distributions

To contrast the geographic distributions of *A. rufopyriformis* and *A. angustilimbatus*, records of these species from our collections were consolidated with those from the published literature (Cook, 1955; Mullen, 1976). Additional records were provided from the Canadian National Collection (Ian Smith, Biosystematics Research Centre, Agriculture Canada).

Genetic analyses

Water mites were collected between 1994 and 1996 from temporary ponds in Ontario, Canada (Table 1), using fine nets and sieves (Smith, Cook & Smith, 2001) and identified with published keys (Mullen, 1976; Smith, 1990b; sample sizes provided in Table 1). Individuals were stored live at 4–8°C prior to genetic analysis. Protein electrophoresis was conducted for 15 putative loci using a cellulose acetate medium: AAT (EC 2.6.1.1, three loci), ADH (1.1.1.1), ARK (2.7.3.3), FUM (4.2.1.2, two loci), G3PDH (1.2.1.12), GPI (5.3.1.9, two loci), MDH (1.1.1.37, two loci), PGM (5.4.2.2, two loci) and 6PGDH (1.1.1.44). The protocol was modified from Hebert & Beaton (1993); see Bohonak, 1999 for details. For these markers, Bohonak (1999) summarised diversity within species as H_0, observed (direct-count) heterozygosity. Here, we also report P, the proportion of polymorphic loci (95% criterion) and A, the number of alleles per locus. Each of these three statistics was analysed here at the population (pond) level, as well as at the species level. Averages for each pond were used for statistical comparisons because of different sample sizes in each population, and the assumption that ponds are the most appropriate unit of replication.

Genetic divergence between *A. angustilimbatus* and *A. rufopyriformis* was calculated as Nei's (1972) D. Within each species, divergence among populations (ponds) was calculated by Bohonak (1999) as Weir & Cockerham's (1984) θ (an estimator of Wright's (1931) F_{ST}), which corresponds to a simple island model. Error estimates for θ were determined by jackknifing

Table 1 Sample sizes for morphological and genetic analyses of *Arrenurus angustilimbatus* and *A. rufopyriformis*. Each population corresponds to a single wetland site (e.g. pond, bog or seepage area)

Mite species	Host	Mite population	Number of analysed specimens	
			Morphology	Genetics
A. rufopyriformis	None	BC	36	58
		CS	57	101
		MB	36	53
		MD	39	79
		DH	33	44
A. angustilimbatus	Mosquitoes (*Aedes* spp.)	LM	58	42
		MS	48	38
		T1	67	46
		T2	18	34
		T4	25	68
		T5	20	15
		TC	73	17

across loci, and from 500 bootstraps conducted across loci for each species (Weir, 1990). Here, we compare genetic divergence using a more detailed analysis of 'isolation by distance', which adjusts for the spatial scale of sampling (Slatkin, 1993; Bohonak, 2002). For these analyses, the statistic $\hat{M} = (1 - \theta)/4\theta$ was calculated for all pairwise combinations of populations within each species. Patterns of isolation by distance were determined from bivariate plots of log ($\hat{M}$) versus log (geographical distance), using population pairs as points. These particular metrics were chosen because theoretical studies have demonstrated their utility in uncovering different patterns and rates of gene flow in spatially explicit models (see Slatkin, 1993).

Morphological analyses

Live *A. angustilimbatus* and *A. rufopyriformis* adults were collected from temporary ponds throughout southeastern and central Ontario in April and May of 1995 for morphological analysis. In the laboratory, each adult females was placed in a 4.7 cm high × 2 cm diameter glass shell vials three-fourths filled with pond or lake water, fed *Cyprinis pubera* ostracods, and monitored for egg production. Images of adult females and their eggs from a microscope-mounted Panasonic BL200 black and white video surveillance camera were captured using an AgVision image capture board and AgImage software v. 1.08 (Decagon Devices, Pulman, WA, U.S.A.).

After electronically editing the legs from the image, the dorsal area of each female (body area) was calculated using the automated image area function of the AgImage programme. Contrast was not sufficient to capture the measurement of the gonopore directly, so mouse-controlled length measurement was used to measure gonopore length and width from the image. Gonopore area was then calculated using the average of the length and width as the diameter for the area of a circle. Egg area was calculated by first using the automated average radius routine, and using this measurement to calculate the profile area of the egg. Eggs were very close to spherical, but adult females were slightly elongate (depth representing approximately 2/3 length). Although volume might represent a better metric of body or egg size, body and egg area are more comparable with gonopore area, and estimating the depth of each specimen added significantly to the effort.

Gonopore area and egg area are expected to correlate with body area due to simple allometric relationships. It is also possible that these relationships might differ among species. We used analysis of covariance (ANCOVA) to test the hypothesis that allometric relationships differ between species, with gonopore area or egg area as the dependent variable, body area as the covariate and species as a categorical independent variable. Interactions between body area and species were included in the model to determine whether the slope of these relationships is species-dependent.

Our primary goal was to test for morphological differentiation among populations and species. To achieve this, we first tested for differences in body area among populations (regardless of species) using

ANOVA, with population as a fixed independent factor. (Log transformation of body area was necessary to meet normality assumptions.) Second, we tested for differences among populations in gonopore size, after reducing biases caused by allometry. This second ANOVA used residuals from the ANCOVA described above as a dependent variable, and population as the fixed independent factor. Significant results for both ANOVAs were assessed using Scheffe's *post hoc* test for all possible population pairs (Neter, Wasserman & Kutner, 1990).

Finally, we examined morphological divergence among populations in a framework comparable with the isolation by distance analyses used for the genetic data. We first calculated the mean value of log(body area) for each population. For all pairs of populations within the two species, the difference between the means was then plotted against geographic distance and examined visually. All statistical analyses were performed on a G4 Macintosh computer using DataDesk v. 6.1.1 (Velleman, 1997).

Results

Species distributions

The known distributions of *A. angustilimbatus* and *A. rufopyriformis* differ in a manner consistent with their life histories (Fig. 1). *Arrenurus angustilimbatus* is widespread. Twenty-nine populations have been recorded across all of southern Canada and the northern U.S.A, with an additional geographic outlier in Yukon Territory, near the Alaskan border. Because sampling efforts have been extremely limited outside of Ontario and upstate New York, it seems likely that *A. angustilimbatus* is found in much of the suitable habitat throughout this range, probably even including the northern tundra. In contrast, only 10 populations of the direct developer *A. rufopyriformis* have been reported. In Ontario, repeated sampling by ourselves and others over the past two decades has yielded only five populations, four of which are within 23 km of each other near Ottawa (Fig. 1). The *A. rufopyriformis* populations located outside of Ontario consist of the type locale in New Jersey, three ponds in Michigan and a single individual from Wyoming (Cook, 1955; Mullen, 1976). We question the record of *A. rufopyriformis* from Wyoming, because it represents a single individual taken from a fish stomach and collected prior to the description of *A. angustilimbatus.* We have not had the opportunity to examine this specimen.

Genetic analyses

Allozyme analysis revealed that *A. angustilimbatus* and *A. rufopyriformis* are very similar genetically

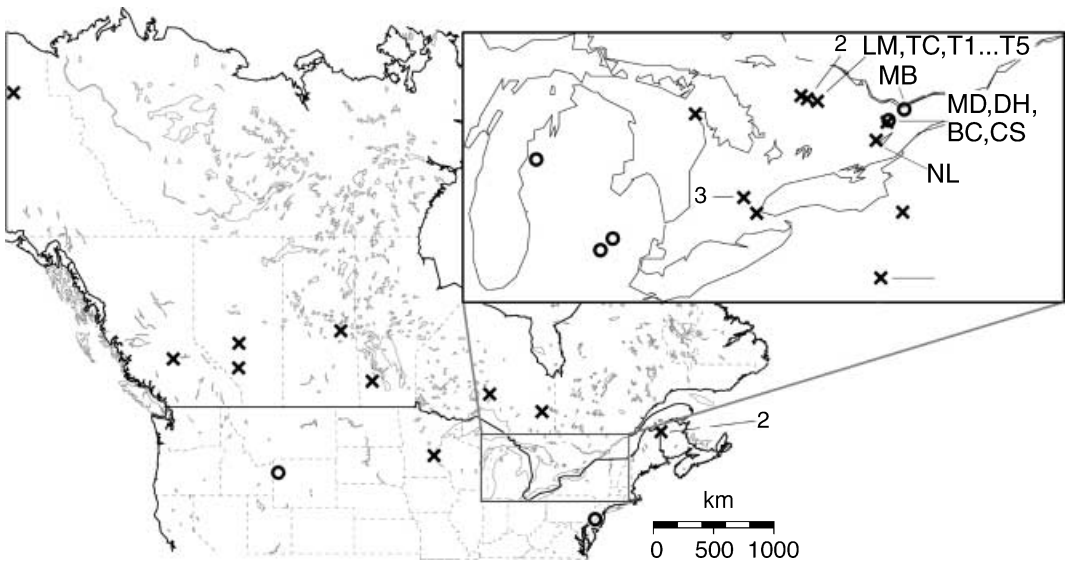

Fig. 1 Distribution of *Arrenurus angustilimbatus* (crosses) and *A. rufopyriformis* (circles). Numbers indicate multiple populations in close proximity.

(Table 2). Beyond a complete absence of fixed differences between these species, frequencies at each locus were nearly identical and the only examined alleles unique to one species were those with frequencies ≤0.013. Genetic distance between these species is extremely low (Nei's $D = 0.002$).

As reported by Bohonak (1999), observed heterozygosity in the direct developer *A. rufopyriformis* was nearly 20% lower than in *A. angustilimbatus* (median $H_0 = 0.087$ and 0.107; Fig. 2). Only the *A. rufopyriformis* MB population showed greater heterozygosity than the lowest *A. angustilimbatus* population. This difference was significant whether comparisons were made at the level of populations ($P < 0.05$, Mann–Whitney *U*-test) or loci ($P < 0.05$, one-sided sign test). Neither the proportion of polymorphic loci nor the number of alleles per locus was statistically different between species ($P = 0.87$ and 0.81, respectively).

Table 2 Allele frequencies at eight variable allozyme loci in *Arrenurus angustilimbatus* (average sample size per locus = 191 individuals) and *A. rufopyriformis* (average sample size per locus = 258)

Locus	Allele	*A. rufopyriformis*	*A. angustilimbatus*
AAT-1	1	0.024	0.009
	2	0.952	0.964
	3	0.024	0.027
AAT-2	1	0.003	0.020
	2	0.033	0.074
	3	0.915	0.847
	4	0.023	0.059
	5	0.010	
	6	0.013	
	7	0.003	
ADH	1	0.002	0.002
	2	0.998	0.996
	3		0.002
ARK	1	0.002	0.018
	2	0.997	0.982
	3	0.002	
FUM-2	1	0.045	0.008
	2	0.535	0.498
	3	0.362	0.405
	4	0.056	0.089
	5	0.002	
GPI-1	1	0.006	0.002
	2		0.002
	3	0.004	0.023
	4	0.985	0.952
	5	0.004	0.017
	6		0.004
MDH-1	1	0.012	
	2	0.978	0.996
	3	0.010	0.004
PGM-1	1	0.003	0.014
	2	0.006	0.006
	3	0.114	0.077
	4	0.042	0.071
	5	0.571	0.482
	6	0.002	0.006
	7	0.250	0.311
	8	0.011	0.004
	9	0.002	0.028
	10		0.002

Population differentiation was very low in both water mite species based on allozymes, but slightly higher in *A. rufopyriformis* ($\theta = 0.032$) than in *A. angustilimbatus* ($\theta = 0.012$) (Bohonak, 1999). In both cases, θ was significantly greater than zero when error estimates were obtained from bootstrapping or jackknifing over loci ($P < 0.05$). Statistical significance for the slight difference between species was found when error estimates were derived from jackknifing over loci ($P < 0.05$), but not for bootstrapping (Bohonak, 1999). Inspection of geographic patterns at the population level demonstrated that these contrasts were not distance-dependent (Fig. 3). Pairs of *Arrenurus* populations 300 m apart were as genetically distinct as populations almost 200 km apart. Fig. 3 also demonstrates that the lower estimate of θ in *A. angustilimbatus* is not a statistical artefact of different sampling scales for the two species.

Morphological analyses

Both gonopore area and egg area were correlated with body area (Fig. 4), and the nature of this relationship was species-dependent. For example, individual *A. rufopyriformis* tend to have a larger gonopore area for any particular body size ($P < 0.0001$ for ANCOVA species term), although the regression slope does not differ statistically between the two species ($P = 0.12$ for species × body area interaction). The dependence of egg area on body area is similar, with statistically significant differences for species intercepts but not for slopes (ANCOVA, $P < 0.0001$ and $P = 0.06$, respectively).

Body areas were not the same in all populations (ANOVA on log(body area), $P < 0.0001$), and *post hoc* contrasts between populations in different species were all highly significant ($P < 0.0001$). Four of 31 contrasts between populations of the same species were also significant ($P < 0.05$). The smallest two populations of *A. rufopyriformis* differed significantly

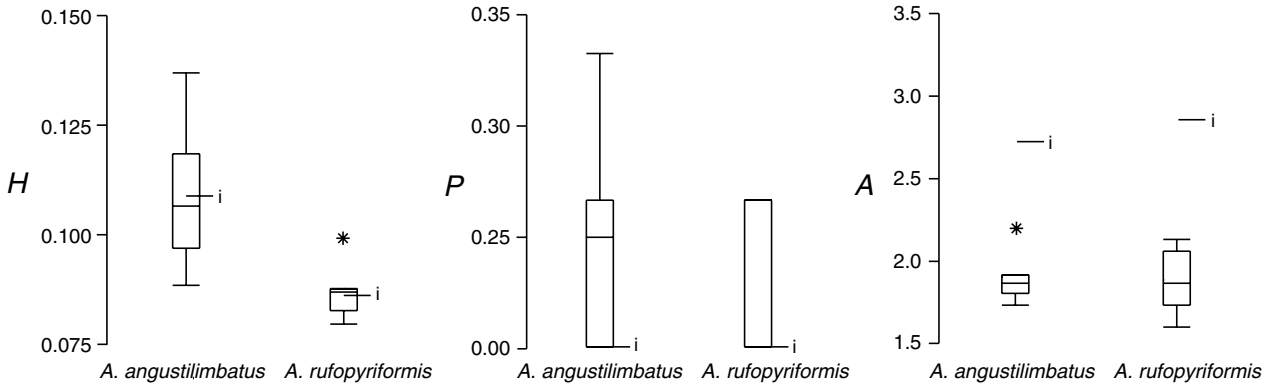

Fig. 2 Genetic diversity of populations within each species, summarised as heterozygosity (*H*), proportion of polymorphic loci (*P*) and number of alleles per locus (*A*). Box plots of each variable depict the median, 25th and 75th percentiles (box hinges), whiskers extending an additional 1.5 × (high hinge – low hinge), and outliers (stars) (Velleman, 1997). *H*, *P* and *A* estimated at the individual level (ignoring populations) are indicated with *i*.

from the largest two populations (Fig. 5). Size-adjusted gonopore area also varied among populations (ANOVA on residuals from above ANCOVA, $P < 0.0001$). For this morphological character, Scheffe's *post hoc* contrasts were not able to reveal which populations contributed to this result (all $P > 0.2$).

The spatially explicit analysis of body area demonstrated that morphologically, pairs of *A. rufopyriformis* populations diverged more rapidly with geographic distance than *A. angustilimbatus* populations pairs (Fig. 6). With one exception, divergence between *A. rufopyriformis* population pairs exceeded 9% of body area (after log transformation, equal to 0.04), and reached a maximum of 50% divergence (see also Fig. 5). In contrast, 14 of 21 pairs of *A. angustilimbatus*

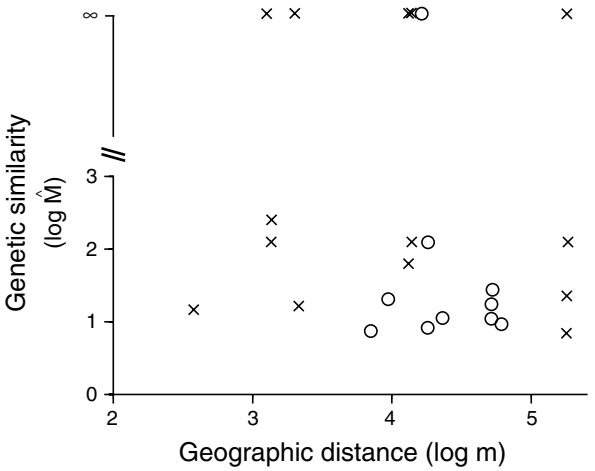

Fig. 3 Genetic similarity as a function of geographic distance for all pairwise combinations of *A. angustilimbatus* populations (crosses) and *A. rufopyriformis* populations (circles). $\hat{M}$ is set at its theoretical maximum of infinity for $\theta \leq 0$.

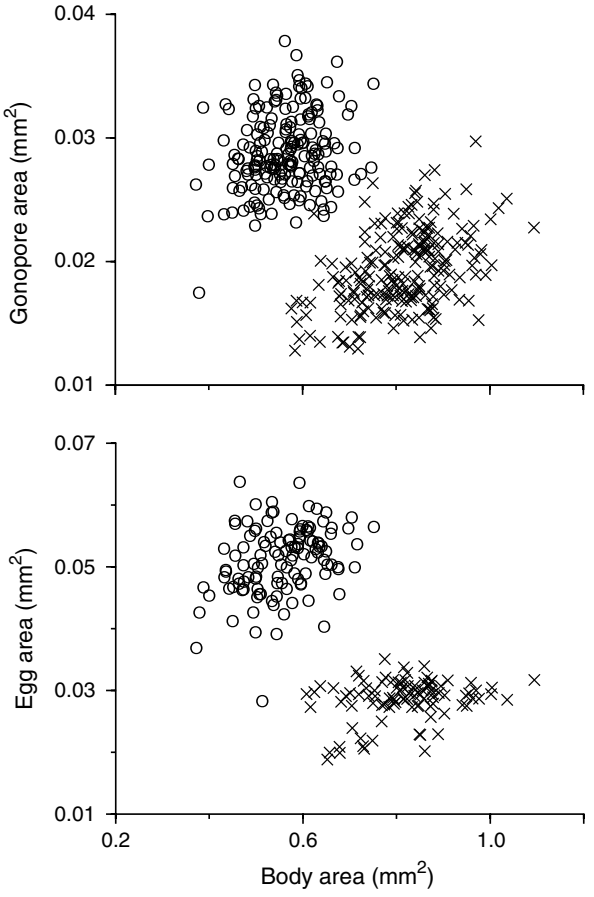

Fig. 4 Gonopore area and egg area as a function of body area. Individual *Arrenurus angustilimbatus* are indicated with crosses, and *A. rufopyriformis* with circles.

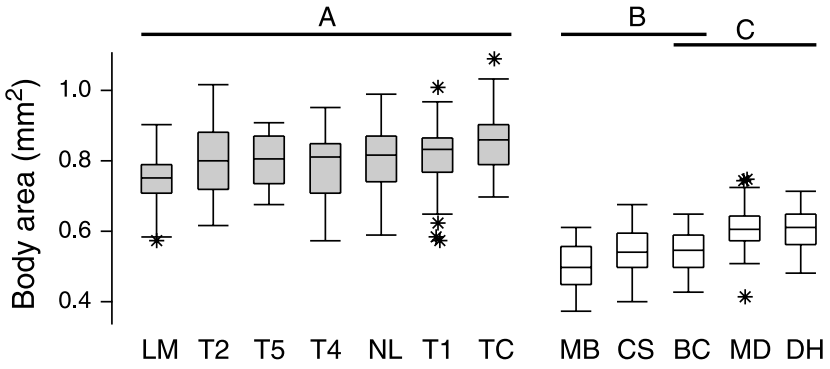

Fig. 5 Box plot of body area across populations (symbols as in Fig. 2). Boxes for *Arrenurus angustilimbatus* are shaded, and boxes for *A. rufopyriformis* are open. Letters designate groups of ponds that are not significantly different in *post hoc* tests from an ANOVA of log (body area) across populations (see text for details).

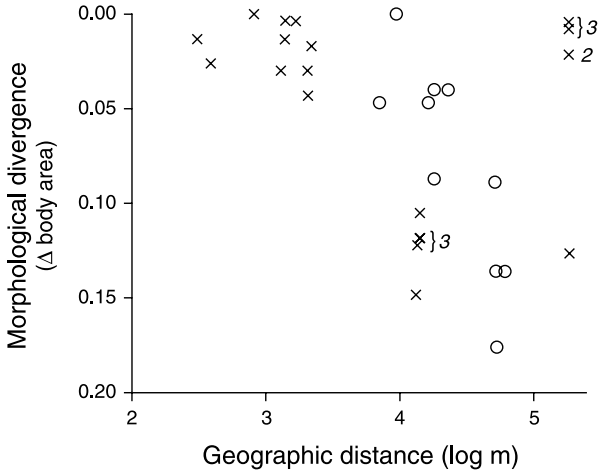

Fig. 6 Morphological divergence as a function of geographic distance for all pairwise combinations of *Arrenurus angustilimbatus* populations (crosses) and *A. rufopyriformis* populations (circles). Divergence is calculated as the difference in mean (log-transformed body area) between populations. Note that the scale on the ordinate is inverted to facilitate comparison with Fig. 3.

populations were less than 9% divergent. The trend towards increasing morphological divergence over larger distances in *A. rufopyriformis* (Fig. 6) contrasted with patterns of genetic divergence over the same populations scale (Fig. 3). Both the analysis of variance and the isolation by distance plot demonstrated that population structure in this species is more pronounced for body size than genetic markers.

Discussion

Multiple lines of evidence suggest that dispersal via insects is a highly influential life-history characteristic for the temporary pond water mite *A. angustilimbatus*. This species possesses a greater degree of genetic cohesion and range continuity than its sister species which does not disperse on adult mosquitoes. If 'ecological success' is measured by the number of populations in a species or its geographic range, it is clear that *A. angustilimbatus* is more successful than *A. rufopyriformis*. Because evolutionary potential is often equated with genetic variation (e.g. Frankham, 1993), higher heterozygosity in *A. angustilimbatus* is also consistent with greater evolutionary success. It is less clear whether the relative independence that populations of *A. rufopyriformis* experience because of reduced gene flow (Figs 3, 5 & 6) is advantageous or detrimental. Although low rates of gene flow may slow the spread of favourable mutations, reduced gene flow can also facilitate local adaptation and the formation of new species (Slatkin, 1987).

Attempts to delineate the range of these species must be qualified by addressing appropriate caveats. Sampling effort for temporary pond water mites has been very limited in North America, even more than other major groups of temporary pond arthropods. A relative dearth of acarologists has biased collecting efforts regionally and in terms of the habitats surveyed. As a result, our confidence in the distributions of these species declines from east to west in parallel with the amount of sampling effort. Despite these limitations, some generalisations are possible. Neither *A. angustilimbatus* nor *A. rufopyriformis* are very common, probably because they have relatively strict habitat requirements. Survival in these species requires an adequate supply of microcrustacean prey during the active adult stage, and water-saturated conditions in thick moss or wet mud throughout the autumn and winter. (Temporary pond *Arrenurus* in the subgenus *Truncaturus* are unable to survive complete desiccation.) It is also evident that *A. angustilimbatus* is more abundant and has a more continuous distribution than its direct-developing

sister species. Evolutionary relationships among the three regional *A. rufopyriformis* centres (Ottawa, New Jersey and Michigan) require further investigation. For example, DNA sequence information could be used to verify that *A. rufopyriformis* (as currently recognised) is monophyletic with respect to *A. angustilimbatus*, and to infer divergence times among the three regions.

Several interpretations of the distributional differences are possible. If it is assumed that temporary pond animals require dispersal in space or time in order to persist through particularly dry years (Williams, 1998), then the ecological success of *A. angustilimbatus* may directly reflect its ability to disperse on insects. Variation in pond hydrology (e.g. Bonis, 1998) is expected to affect survivorship negatively during both periods when ponds are filled with water (because of decreased prey abundance) and when standing water is lacking. In a metapopulation where individual pond populations may experience extinctions, *A. angustilimbatus* will succeed in colonising new habitats more often (and at greater distances) than *A. rufopyriformis*. In conjunction with a balance between extinction and limited colonisation potential, centres of regional endemism for *A. rufopyriformis* may also include populations which are extremely stable over geologic time. In these ponds, established populations of *A. rufopyriformis* could outcompete invading *A. angustilimbatus*, because *A. rufopyriformis* does not need a host to complete its life cycle, and can thus have multiple generations annually. In contrast, synchronisation with the life cycle of its hosts (*Aedes* spp. mosquitoes) dictates a single generation per year for *A. angustilimbatus* (Smith & McIver, 1984b). *Arrenurus angustilimbatus* is also expected to experience increased mortality during the larval stage because of risks inherently associated with parasitism. These include the risk of not locating a host, of being killed by the host immune response and of not returning to a suitable habitat (see Smith & McIver, 1984a).

Results of the genetic and morphological analyses were largely congruent with the distributional data. Population-level contrasts for *A. angustilimbatus* revealed genetic and morphological uniformity over spatial scales that vary from 300 m to 185 km. Population differentiation (estimated as θ) is very close to zero, and is not dependent on the distance between populations. The lack of isolation by distance over large spatial scales (Fig. 3) is consistent with a departure from drift-gene flow equilibrium, such as recent (post-glaciation) range changes or a complex metapopulation structure (Wade & McCauley, 1988; Slatkin, 1993; Bohonak, 1999; Bohonak & Roderick, 2001). Although patterns of genetic structure in the direct developer *A. rufopyriformis* are qualitatively similar, levels of divergence are slightly higher. This suggests a role for insect-mediated dispersal in maintaining genetic panmixia (complete mixing) in *A. angustilimbatus*. The importance of dispersal can be generalised to explain morphological uniformity among populations of *A. angustilimbatus* but not *A. rufopyriformis*, and to explain the higher heterozygosity found in *A. angustilimbatus*. All other factors being equal, decreased gene flow in *A. rufopyriformis* should increase local inbreeding, and decrease the amount of genetic variation that is maintained.

These comparisons are dependent on assumptions about the taxonomy and systematics of *A. angustilimbatus* and *A. rufopyriformis* that are supported by morphology, breeding experiments and species distributions, but untested using DNA sequences. Unfortunately, the allozyme data provide little guidance in this area because of low resolution. Very low Nei's *D* and an absence of fixed differences between species are consistent with either a very recent origin for *A. rufopyriformis*, or with ongoing hybridisation between the supposed species. The life history of *A. angustilimbatus* makes it likely that if hybridisation does occur, it is asymmetric. Large populations of *Aedes* spp. mosquitoes (the typical host of *A. angustilimbatus*) are found in habitats of both species, and ponds can be found in close enough proximity that the possibility of unidirectional hybridisation should be considered. Under this scenario, the active transfer of *A. angustilimbatus* genes into *A. rufopyriformis* populations must be balanced by strong natural selection, because morphological differentiation among the two species is disjunct (Fig. 4). Further studies utilising rapidly evolving, genealogically informative molecular markers may help to resolve these issues, as will work on other directly developing water mites.

Acknowledgments

The authors would like to thank David Jenkins and Steven Schwartz for organising an ASLO symposium

in 2001 in which this paper was presented, and for their editorial guidance. Dr Ian Smith provided advice and access to specimens and records held in the Canadian National Collection. Kit Muma and Christina Coleman helped with field collections and maintaining mites in the laboratory. The Ontario Ministry of Natural Resources, Queen's University Biology Station and the Wildlife Research Station in Algonquin Provincial Park provided lodging, laboratory facilities and access to field sites. AJB was supported by a National Science Foundation grant (DEB-9423603) and a grant from San Diego State University, and MT was supported by the Ford Fund.

References

Bohonak A.J. (1999) Effect of insect-mediated dispersal on the genetic structure of postglacial water mite populations. *Heredity*, **82**, 451–461.

Bohonak A.J. (2002) IBD (Isolation By Distance): a program for analyses of isolation by distance. *Journal of Heredity*, **93**, 153–154.

Bohonak A.J. & Jenkins D.G. (2003) Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology Letters*, **6**, 782–796.

Bohonak A.J. & Roderick G.K. (2001) Dispersal of invertebrates among temporary ponds: are genetic estimates accurate? *Israel Journal of Zoology*, **47**, 367–386.

Bonis A. (1998) Coexistence in a variable and unpredictable habitat: the importance of the seed bank and contrasted regeneration niche for annual plants in temporary pools. *Archiv für Hydrobiologie – Advances in Limnology*, **52**, 53–69.

Brock M.A., Nielsen D.L., Shiel R.J., Green J.D. & Langley J.D. (2003) Drought and aquatic community resilience: the role of eggs and seeds in sediments of temporary wetlands. *Freshwater Biology*, **48**, 1207–1218.

Cook D.R. (1954) Preliminary list of the Arrenuri of Michigan Part II. The subgenus *Megaluracarus*. *Transactions of the American Microscopical Society*, **73**, 367–380.

Cook D.R. (1955) Preliminary list of the Arrenuri of Michigan Part III. The subgenera *Micruracarus* and *Truncaturus*. *Transactions of the American Microscopical Society*, **74**, 60–67.

Cook D.R. (1974) *Water Mite Genera and Subgenera. Memoirs of the American Entomological Institute. No. 21*. American Entomological Institute, Ann Arbor, MI.

Cook W.J., Smith B.P. & Brooks R.J. (1989) Allocation of reproductive effort in female *Arrenurus* spp. water mites (Acari: Hydrachnidia; Arrenuridae). *Oecologia*, **79**, 184–188.

Frankham R. (1993) Conservation genetics. *Annual Review of Genetics*, **29**, 305–327.

Habeeb H. (1954) North American Hydrachnellae, Acari XVIII-XXV. *Leaflets of Acadian Biology*, **4**, 1–8.

Hairston N.G. Jr (1996) Zooplankton egg banks as biotic reservoirs in changing environments. *Limnology and Oceanography*, **41**, 1087–1092.

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

Harvey P.H. & Pagel M.D. (1991) *The Comparative Method in Evolutionary Biology*. Oxford University Press, New York.

Hebert P.D.N. & Beaton M.J. (1993) *Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis: A Practical Handbook*. Revised edn. Helena Laboratories, Beaumont, TX.

Lundblad O. (1941) Neue Wassermilben aus Amerika, Afrika, Asien und Australien. *Zoologischer Anzeiger*, **133**, 155–160.

Maguire B. Jr (1963) The passive dispersal of small aquatic organisms and their colonization of isolated bodies of water. *Ecological Monographs*, **33**, 161–185.

Marshall R. (1903) Ten species of Arrenuri belonging to the subgenus *Megalurus* Thon. *Transactions of the Wisconsin Academy of Sciences, Arts, and Letters*, **14**, 145–173.

Mullen G.R. (1976) *Water Mites of the Subgenus Truncaturus* (Arrenuridae, *Arrenurus*) in North America, Vol. 6. Cornell University Agricultural Experiment Station Bulletin, Ithaca, NY.

Nei M. (1972) Genetic distance between populations. *American Naturalist*, **106**, 283–292.

Neter J., Wasserman W. & Kutner M.H. (1990) *Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs*, 3rd edn. Irwin, Homewood, IL.

Peck S.B. (1975) Amphipod dispersal in the fur of aquatic mammals. *Canadian Field-Naturalist*, **89**, 181–182.

Simovich M.A. & Hathaway S.A. (1997) Diversified bet-hedging as a reproductive strategy of some ephemeral pool anostracans (Branchiopoda). *Journal of Crustacean Biology*, **17**, 38–44.

Skelly D.K. (1996) Pond drying, predators, and the distribution of *Pseudacris* tadpoles. *Copeia*, **1996**, 599–605.

Slatkin M. (1987) Gene flow and the geographic structure of natural populations. *Science*, **236**, 787–792.

Slatkin M. (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

Smith B.P. (1988) Host-parasite interaction and impact of larval water mites on insects. *Annual Review of Entomology*, **33**, 487–507.

Smith B.P. (1990a) Descriptions of larval *Arrenurus bartonensis* Cook, *Arrenurus birgei* Marshall, *Arrenurus neobirgei* Cook, and *Arrenurus rotundus* Marshall (Acari: Hydrachnidia; Arrenuridae). *Canadian Entomologist*, **122**, 77–92.

Smith B.P. (1990b) Hydrachnidia. In: *Freshwater Macroinvertebrates of Northeastern North America* (Eds B.L. Peckarsky, P.R. Fraissinet, M.A. Penton & D.J. Conklin, Jr, pp. 290–334. Comstock Publishing/Cornell University Press, Ithaca, NY.

Smith B.P. (1998) Loss of larval parasitism in Parasitengonine mites. *Experimental and Applied Acarology*, **22**, 187–199.

Smith B.P. & McIver S.B. (1984a) Factors influencing host selection and successful parasitism of *Aedes* spp. mosquitoes by *Arrenurus* spp. mites. *Canadian Journal of Zoology*, **62**, 1114–1120.

Smith B.P. & McIver S.B. (1984b) The patterns of mosquito emergence (Diptera: Culicidae; *Aedes* spp.): their influence on host selection by parasitic mites (Acari: Arrenuridae; *Arrenurus* spp.). *Canadian Journal of Zoology*, **62**, 1106–1113.

Smith I.M. & Oliver D.R. (1986) Review of parasitic associations of larval water mites (Acari: Parasitengona: Hydrachnida) with insect hosts. *Canadian Entomologist*, **118**, 407–472.

Smith I.M., Cook D.R. & Smith B.P. (2001) Water mites. In: *Ecology and Classification of North American Freshwater Invertebrates* (Eds. J.H. Thorp & A.P. Covich), pp. 523–592. Academic, New York.

Velleman P.F. (1997) *DataDesk for Data Description*. Ithaca, NY.

Viets K.O. (1987) *Die Milben des Süsswassers (Hydrachnellae und Halacaridae [part.], Acari). 2: Katalog. Sonderbände des Naturwissenschaftlichen Vereins in Hamburg 8*. Verlag Paul Parey, Hamburg.

Wade M.J. & McCauley D.E. (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.

Weir B.S. (1990) *Genetic Data Analysis: Methods for Discrete Population Analysis*. Sinauer Associates, Sunderland, MA.

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

Williams D.D. (1998) The role of dormancy in the evolution and structure of temporary water invertebrate communities. *Archiv für Hydrobiologie – Advances in Limnology*, **52**, 109–124.

Wright S. (1931) Evolution in Mendelian populations. *Genetics*, **16**, 97–159.

(Manuscript accepted 3 November 2003)