

Ephemeral metapopulations show high genetic diversity at regional scales

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Abstract. One of the primary questions concerning the long-term preservation of nature and its diversity is the maintenance of genetic diversity. However, despite numerous theoretical investigations, comparative empirical information on how local extinctions influence regional genetic variation does not exist. To our knowledge, this is the first report of an empirical study comparing the genetic variation of permanent vs. ephemeral species at two scales (local variation, regional variation). This approach, utilizing a microsatellite analysis of six midge species of the genus *Chaoborus* generated intriguing scale-dependent results. Species that experienced repeated local extinctions had reduced genetic variation at the local level, yet the regional genetic variation was greater than in species with permanent populations. Our findings call into question the assumption that species with repeated local extinctions generally contain lower genetic diversity, especially if they experience a “nomadic” pattern of dispersal. We encourage comparative analyses of empirical genetic data at dual scales as molecular tools become more available in ecological studies.

Key words: *Chaoborus*; ephemeral; extinction; genetic diversity; local diversity; metapopulation; midges; regional diversity.

INTRODUCTION

How to maintain biodiversity is a primary concern not only for scientists, but also for wildlife management and politicians. One of the most important aspects of local extinctions is that genetic information will be lost, and eventually, will have fitness effects that lead to the extinction of the species (Saccheri et al. 1998). However, despite interest in the maintenance of species-wide genetic variation, no field studies of genetic variation have contrasted local and regional genetic variation between similar species that possess contrasting life histories. Comparative empirical data is almost completely lacking about whether there is reduced regional genetic variation in species that occupy ephemeral habitats, i.e., that experience frequent local extinctions (Hanski 1998). The majority of studies involve instances of recent anthropogenic fragmentation, but empirical studies from ecologically well-characterized natural metapopulations are needed to derive general rules about the contribution of migration and demography to the pattern of genetic variation in naturally extinction-prone metapopulations (Aars et al. 2006).

One of the best-known concepts that potentially addresses ephemeral existence is the metapopulation concept (Hanski 1998). In its broadest definition, a metapopulation is a population of local populations or demes. Generally, these metapopulation models suggest that genetic diversity will depend heavily on the degree to which dispersers share a common origin, as well as on the rates of extinction and recolonization (Pannell and Charlesworth 1999). In most cases, subdivision of the range or extinctions of local populations are likely to induce some loss of genetic variability, and the magnitude will be heavily dependent on how subdivision impacts migration or variance in reproductive success.

Here, we contrast patterns between species that occupy permanent vs. ephemeral habitats, using species of the genus *Chaoborus* (phantom midge), a cosmopolitan insect genus in the order Diptera that has a dual life history. The planktonic larvae of the phantom midge *Chaoborus* live only in freshwater systems, whereas the adults are winged and disperse by flight (Fig. 1A). We focused on six species: *Chaoborus punctipennis* (Say 1823), *C. astictopus* (Dyar and Shannon 1924), *C. americanus* (Johannsen 1903), *C. crystallinus* (De Geer 1776), *C. obscuripes* (Van der Wulp 1859), and *C. flavicans* (Meigen 1830). The ecology of these species is exceptionally similar, except for local population size and a few important aspects of their life-style. There is an interesting difference in their ecological preferences, as species like *C. flavicans*, *C. punctipennis*, and *C.*

Manuscript received 10 April 2008; revised 4 December 2008; accepted 5 February 2009; final version received 17 April 2009.
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astictopus are able to coexist with fish, and therefore live mainly in lakes. The other species, *C. crystallinus*, *C. obscuripes*, and *C. americanus*, cannot coexist with fish and live only in small fish-free pools (Borkent 1981, Von Ende 1982, Berendonk and Bonsall 2002). The difference in the ability to coexist with fish has an interesting consequence for geographic population structure, because it has been shown that the species that live in lakes exist in large populations that rarely go extinct (“permanent species”), whereas species that live in fish-free water bodies live in comparatively small populations that frequently go extinct (“ephemeral species” [Von Ende 1982]). While the demographic data is best documented for *C. flavicans* and *C. crystallinus*, this pattern of population structure has been observed for all the above-mentioned species (e.g., Borkent 1981, Garcia and Mittelbach 2008). It is important to note that all *Chaoborus* species have no resting stages, which would allow them to survive unfavorable conditions in a pond or lake (Borkent 1981, Von Ende 1982, Berendonk and Bonsall 2002).

Generally, local population sizes vary considerably for both types of *Chaoborus* species, although population size is two orders of magnitude larger for local populations of the permanent species than for the ephemeral species. The differences are probably due mainly to the larger habitats (lakes), compared to the relatively small size of ephemeral pools. Both types of species can reach high population densities quickly (Wissel et al. 2000). Also, the population density of the ephemeral species in a given region can be up to two orders of magnitude higher than the population density of the permanent species (Davies et al. 2008). The higher number of populations of the ephemeral species is almost compensated by the larger local population sizes of the permanent species. Therefore, the regional population size is roughly comparable within an order of magnitude for both types of species, but the distribution of the local population sizes and number of populations within regions is very different.

The striking difference in life history between the two groups of *Chaoborus* species allows a comparative test on how population persistence and dispersal will affect genetic variation of a species at multiple scales (local, regional). Given a similar regional population size, genetic variation is mainly influenced by dispersal ability and genetic composition of colonizers. Here genetic data are used to make inferences about dispersal and colonization processes and to test several contrasting hypotheses. (1) For species living in ephemeral habitats, the levels of regional and local genetic variability will both be reduced when the dispersal ability is insufficient to counteract genetic drift caused by high local extinction rates and high variance in reproductive success. (2) Conversely, the alternative hypothesis postulates that the ephemeral nature of the habitats will have no effect on the (relative) levels of genetic variability of these species, when dispersal ability is

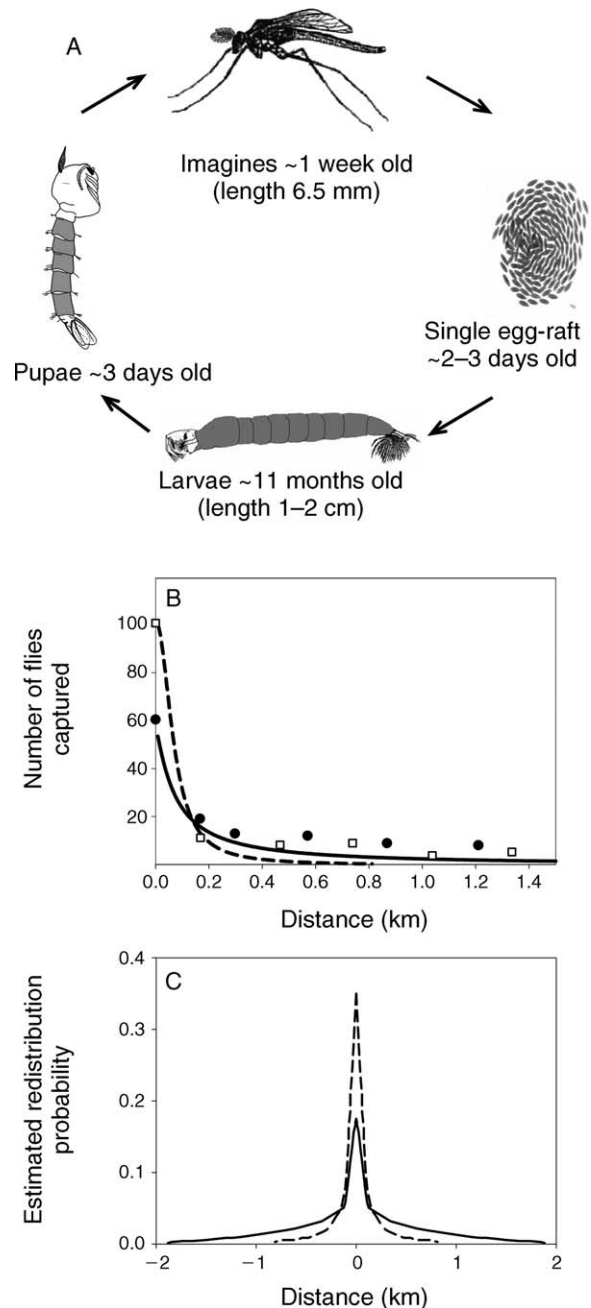


FIG. 1. *Chaoborus* in permanent and ephemeral habitats. (A) Life cycle of *Chaoborus*, illustrating the various stages (egg, larval, pupal, adult). (B) The dispersal curves show the number of female imagines that have oviposited at different distances from the source populations for *C. crystallinus* (ephemeral, solid line and solid circles) and *C. flavicans* (permanent, dashed line and open squares). (C) The redistribution kernels for *C. crystallinus* (ephemeral, solid line) and *C. flavicans* (permanent, dashed line) are shown. The figure is modified from Berendonk and Bonsall (2002).

sufficient to counteract genetic drift and the variance in reproductive success. However, taking into account the composition of the colonizers, two additional hypotheses are possible. (3) The level of regional genetic variability will be (relatively) reduced, but the local genetic variability will be high, when dispersal is moderate and colonization occurs via several individuals from within the region (no long-distance dispersal, colonizers are genetically dissimilar). (4) The (relative) level of regional genetic variability will not be reduced, but the local genetic variation will be low when dispersal is high, yet only a few individuals from outside the region colonize local habitats (frequent long-distance dispersal, colonizers are genetically diverse).

MATERIAL AND METHODS

For each *Chaoborus* species, a total of eight populations with 24 individuals per site were sampled. Four populations of these eight were obtained in two different regions. The samples were taken within a radius of 50 km for all regions. The approximate geographic centers of the sampled regions and the distances among the regions are shown in Appendix A: Table A1. *C. flavicans*, *C. obscuripes*, and *C. crystallinus* (see Plate 1) were sampled in northern and southern Europe, whereas *C. punctipennis* and *C. americanus* were sampled in northern and southern North America (USA). For *C. astictopus*, only three populations per region were sampled in California (USA), as this species has a very narrow geographic range (Borkent 1981). Collections for all species were made in the spring before adult emergence, when 100% of the populations were confined to ponds or lakes as over-wintering larvae. Hence, 100% of the genotypes in the populations were available as larvae for sampling.

For each of the *Chaoborus* species, we identified six microsatellite loci and performed a total of 6624 genotyping reactions. (See Appendix A for details.) To estimate different aspects of genetic variation in these species, the average expected heterozygosity within local random mating populations of each species (H_S , local population heterozygosity) was calculated and the regional average expected heterozygosity of each species (H_T , regional heterozygosity) was calculated. Furthermore, we calculated the average number of alleles per local population for each species (A_S , local population allele number) and the regional average number of alleles for each species (A_T , regional allele number). These estimates were determined for each of the six *Chaoborus* species. ARLEQUIN (Excoffier et al. 2005) and GENEPOP 3.2 (Raymond and Rousset 1995) were used to test departures from the Hardy-Weinberg equilibrium and to detect evidence of linkage disequilibrium.

To analyze population differentiation within each species, we performed an analysis of molecular variance (AMOVA) using GENEALX (Peakall and Smouse

2006) and ARLEQUIN (Excoffier et al. 2005; see Appendix A).

For the following statistical analysis, *C. flavicans*, *C. punctipennis*, and *C. astictopus* were grouped as "permanent species," whereas *C. americanus*, *C. obscuripes*, and *C. crystallinus* were grouped as "ephemeral species." To test if local population heterozygosity (H_S) was significantly different between the permanent species and ephemeral species, we employed a nested ANOVA where the populations (as random factor) were nested within the species and the species were nested within their habitat type (permanent or ephemeral). The same approach was used to analyze the within-population allele number (A_S). To test if the regional heterozygosity (H_T) was different between the two groups of species, we employed a Mann-Whitney U test, using the different species within their group as replicates. The same approach was used to analyze the regional allele number (A_T), the genetic differentiation among individuals within populations Φ_{PT} , and the genetic differentiation among populations within regions Φ_{SC} . The differentiation among regions Φ_{RT} was not statistically analyzed, due to the close distance among the *C. astictopus* regions. Additionally, the phylogenetic information available from Berendonk et al. (2003) as well as the CRUNCH algorithm of the phylogenetic independent contrast software package (CAIC; Purvis and Rambaut 1995, Orme et al. 2008) were used to analyze the differences between the species groups for the parameters just described.

RESULTS

There was no evidence of a departure from the expectation of Hardy-Weinberg equilibrium for any of the microsatellite loci, nor did we detect any evidence of linkage disequilibrium for any of the microsatellite loci in all tested species. The analysis of the microsatellite variation suggested that the local population heterozygosity (H_S) and the local population allele number (A_S) of the ephemeral species group was reduced in comparison to the permanent species group (H_S , hierarchical ANOVA, $n = 40$, MS (mean square) species type = 0.16, $F_{1,4} = 24.89$, $P < 0.05$; A_S , hierarchical ANOVA, $n = 40$, MS species type = 9.10, $F_{1,4} = 17.07$, $P < 0.05$; Fig. 2, Local). However, the regional heterozygosity (H_T) was not significantly reduced, and the regional allele number (A_T) was even significantly higher in the ephemeral species compared to the permanent species (H_T , Mann-Whitney U test, $n = 6$, corrected $Z = 0.65$, $P > 0.05$; A_T , Mann-Whitney U test, $n = 6$, corrected $Z = 1.96$, $P < 0.05$, Fig. 2, Regional). Furthermore, the genetic differentiation among individuals within populations (Φ_{PT}) and the genetic differentiation among populations within regions (Φ_{SC}) was higher for the ephemeral species than for the permanent species (Φ_{SC} , Mann-Whitney U test, $n = 6$, corrected $Z = 1.96$, $P < 0.05$; Φ_{PT} , Mann-Whitney U test, $n = 6$, corrected $Z = 1.96$, $P < 0.05$; Table 1). All the above-mentioned trends remained

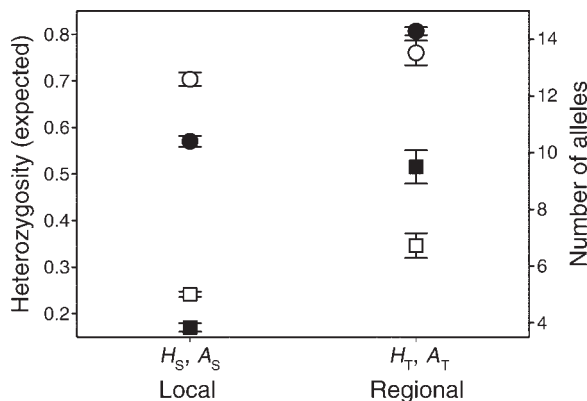


FIG. 2. Comparative genetic diversity for local and regional *Chaoborus* populations. Circles show local (H_S) and regional (H_T) population heterozygosity, and squares show local (A_S) and regional (A_T) population allele numbers for the three ephemeral (solid symbols) and the three permanent (open symbols) *Chaoborus* species. All values are means and standard errors; some error bars are partly obscured by the symbols.

apparent after employing phylogenetic independent contrast analysis (details are shown in Appendix A: Table A2).

DISCUSSION

For *Chaoborus* species that exhibit an ephemeral existence, the local population genetic variation (H_S , A_S ; Fig. 2) is reduced, whereas the regional genetic variation is not (H_T , A_T ; Fig. 2). In fact, the regional allele number is even higher. Additionally, the local populations within regions of the ephemeral species are more different than the local populations of the permanent species (among populations within regions, Φ_{SC} ; Table 1). Therefore, the results indicate that genetic variation in the ephemeral species is not reduced, but differently distributed than in the permanent species. In the ephemeral species the local genetic variation of the populations within regions is low, but populations are genetically very different from each other, and therefore, corresponding to the fourth hypothesis (see *Introduc-*

tion), the regional genetic variation is comparatively high (Fig. 2; Φ_{SC} in Table 1). In contrast, the genetic variation of the local permanent species populations is high, but local populations are genetically much more similar to each other within regions, and therefore the regional genetic variation is comparatively low (Fig. 2; Φ_{SC} in Table 1). Interestingly, despite its small species range, all estimates of the genetic variation for *C. punctipennis* were close to the values of the other permanent species (Appendix A: Table A3).

Theoretical studies of metapopulations suggest that local extinction and colonization per se are expected to lower the effective population size. This is primarily because exchange tends to increase the variance in reproductive success, owing to more successful colonies exporting more colonists to empty patches than less successful ones. If, on the other hand, new colonies are composed of founders that come from many small colonies distributed over a larger area, rather than from a few larger colonies close by, as would be the case in a source-sink system, then the loss of variability could be low relative to that expected in a large panmictic population of the same size (Pannell and Charlesworth 1999, Kaitala et al. 2006).

The results presented here tend to corroborate the hypotheses of the theoretical studies cited previously. The data strongly suggest that colonization of local populations was not performed by just a single or a few neighboring (genetically closely related) gravid females. Table 1 indicates that the individuals of the ephemeral species within a local habitat are more different from each other than the individuals of the permanent species. If in the ephemeral species, the colonization of the local habitats would have occurred via a single or a few gravid neighboring females, the individuals should have been more similar to each other than in the permanent species. The data on the allelic differentiation (Table 1) and diversity (Fig. 2; Appendix B) demonstrate that, while for the ephemeral species the local population genetic variation is lower than for the permanent species, the few local variants within a population are more

TABLE 1. Genetic differentiation of *Chaoborus* midge populations.

Population	Geographic distance among regions (km)	Among individuals within populations, $\Phi_{PT}\dagger$	Among populations within regions, $\Phi_{SC}\dagger$	Among regions, Φ_{RT}
Ephemeral				
<i>C. crystallinus</i>	1264	0.512	0.215	0.378
<i>C. americanus</i>	1476	0.519	0.254	0.355
<i>C. obscuripes</i>	1264	0.474	0.225	0.322
Permanent				
<i>C. flavicans</i>	1397	0.175	0.000	0.175
<i>C. punctipennis</i>	1476	0.222	0.099	0.137
<i>C. astictopus</i>	60	0.098	0.079	0.020

Notes: The table gives geographic distances among regions. It also shows genetic differentiation of populations for six *Chaoborus* species for three different levels: among individuals within populations, among populations within regions, and among regions.

† Significant difference ($P < 0.05$) between ephemeral and permanent groups.



PLATE 1. A male *Chaoborus crystallinus* imago soon after emergence. Photo credit: Malcom Storey.

different from each other for the ephemeral species than for the local variants of the permanent species (see also mitochondrial data in Berendonk and Spitze 2006a, b).

To obtain insight into why local populations of ephemeral species are more differentiated at the regional level, a closer look at the ecology of the permanent and ephemeral species is revealing. In a previous study, Berendonk and Bonsall (2002) focused on the species *C. flavicans* and *C. crystallinus* as representatives of the two groups. They could show that dispersal occurs mainly via inseminated females (Fig. 1A). When they compared the dispersal potential of both species, using direct observations, the two species were similar, except that *C. crystallinus* displayed a selective ovipositioning behavior, avoiding water bodies with fish and consequently dispersing farther than *C. flavicans* (Fig. 1B; Berendonk and Bonsall 2002). Thus *C. flavicans* (permanent species) disperses just to the next available habitat, whereas *C. crystallinus* (ephemeral species) disperses to the next suitable (fishless) habitat, which might often be more distant. This difference in behavior between these species has also been reported for other species pairs of this genus (Garcia and Mittelbach 2008).

Given our observations, greater regional dispersal of females in ephemeral species might be the key variable producing scale-dependent reversals in the *Chaoborus* system. More specifically, the results suggest that a reduction of genetic variation due to local extinctions can be compensated when (a) so many local habitats are occupied that the regional population size is only a few

orders of magnitude smaller than the regional population size of a permanent species, (b) local populations grow fast to high densities, and (c) the dispersal ability of the species allows a movement across the region and therefore a mode of recolonization with founders from many habitats over a larger area. That is, how widespread is the “nomadic” behavior of females that disperse from ephemeral habitats? Is this a dispersal characteristic of ephemeral species?

Our work encourages further investigation of ephemeral vs. permanent genetic patterns and checks for nomadic behavior of females that disperse from ephemeral habitats. For example numerous insect, amphibian, and mollusk species also show a dichotomy between fish-compatible and fish-avoiding species (Kerfoot and Sih 1987, Fraser et al. 2007). Furthermore, many other species groups consist of populations with contrasting persistence, i.e., ciliates with and without encystment (Foissner 2006), or small mammals with strongly differing population dynamics and structures (Brouat et al. 2007). It is important to design the spatial scale of these investigations according to the dispersal ability of the respective species.

If genetic diversity of an ephemeral species is maintained at larger scales despite local extinctions, the pattern has interesting implications for conservation strategies. In ephemeral species, the accumulative contribution of the local population to the regional genetic variation is larger than for species that live in large, permanent populations. Our findings call into

question the assumption that species with repeated local extinctions generally contain lower genetic diversity (Saccheri et al. 1998). A possible implication is that for species that live in fragmented habitats, the number of local populations and the pattern of dispersal are more important for the maintenance of genetic diversity than previously assumed (Ray 2001, Frankham 2003).

ACKNOWLEDGMENTS

We thank J. H. Lawton, H. J. C. Godfray, W. Lampert, M. Whitlock, and J. R. Pannell for project discussions and for consultation on details of the manuscript. T. U. Berendonk thanks the Deutsche Forschungsgemeinschaft (BE 2299/1-1), and W. C. Kerfoot thanks NSF DEB-0083731 (Biocomplexity) for financial support. We also acknowledge A. Borkent for conversations and for help with the sampling. Several scientists (M. Runde, J. M. Schell, M. Bazzanti, M. Manca, R. A. Lillie, and especially N. L. Anderson, Lake County Vector Control, California) aided sampling of *Chaoborus*. We thank most sincerely the Spatial Ecology visitor program of the University of Helsinki, and we enjoyed the hospitality and support of the Hanski group. We are grateful to the University of Notre Dame Environmental Research Center (UNDEC) for their aid in field investigations, as well as Carl N. von Ende for the interesting discussions on *Chaoborus* ecology. Finally, T. U. Berendonk thanks W. Lampert and Sir J. H. Lawton for their continuous support and encouragement.

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APPENDIX A

Additional information for Methods and Results (*Ecological Archives* E090-187-A1).

APPENDIX B

Data on number of alleles (N_a), number of effective alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), and fixation index (F) for each locus for each population and species (*Ecological Archives* E090-187-A2).