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The scale of divergence: A phylogenetic appraisal of intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus

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ABSTRACT

Molecular studies have enlightened our understanding of freshwater zooplankton biogeography, yet questions remain regarding the scale and commonality of geographic speciation. Here, we present a mtDNA-based phylogenetic hypothesis for 92 *Daphnia* species from all seven continents, with a focus on North and South America, Europe, and Australia, and use it to explore the frequency, scale, and geographical orientation of allopatric divergence events. Allopatric speciation can conservatively account for at least 42% of cladogenetic events among the species included in our study; most of these involve intercontinental splits. Closely related species pairs are concentrated in the circumarctic region and between northern and southern continents, aligned with bird migration routes, suggesting recent dispersal. By contrast, deeper phylogenetic patterns are consistent with vicariance scenarios linked to continental fragmentation. The possible reasons for the puzzling persistence of these ancient patterns in light of the eroding force of dispersal are considered. Our results demonstrate the high frequency and complex pattern of allopatric speciation in this ancient, passively dispersed genus.

1. Introduction

A historically close relationship exists between the study of biogeography and our understanding of speciation processes (Darwin, 1859). This association is especially clear within studies of the freshwater invertebrate fauna. The morphological similarity of species inhabiting different continents, combined with observations of strong dispersal mechanisms possessed by freshwater invertebrates, led to the early conclusion of cosmopolitan distributions (Darwin, 1859, 1882; Mayr, 1963). According to this view, global species diversity within many freshwater groups was low, and high dispersal rates limited opportunities for allopatric speciation. Yet detailed morphological (Frey, 1982, 1987) and extensive molecular (e.g. Colbourne et al., 1998; Černý and Hebert, 1999; Gómez et al., 2000; Schwenk et al., 2000; Petrusek et al., 2004; Fontaneto et al., 2008) evidence necessitated a reversal of this view. Despite broad geographic morphological similarities, most aquatic invertebrate species are confined to single continents. Moreover, species richness as well as intraspecific genetic diversity is often high within continents (De Melo and Hebert, 1994; Hann, 1995; Taylor et al., 1998; Cox and Hebert, 2001; Hebert et al., 2003; Penton et al., 2004) and even smaller regions (Hebert and Wilson, 1994, 2000; Kořínek and Hebert, 1996; Kořínek et al., 2003; Petrusek et al., 2007), and has led to a shift towards provincialism as the prevailing biogeographic hypothesis. Concordant with this view, detailed distributional information has revealed that allopatric divergence is likely to be an important mechanism of diversification for invertebrates inhabiting continental waters.

Despite these advances, the relationship between observed distributions and evolutionary processes remains a complex puzzle. The facts that new habitats are rapidly invaded (Louette and De Meester, 2005) and that some genotypes have vast geographic distributions (e.g. Weider et al., 1999a,b) suggest that dispersal potential is high, which is seemingly paradoxical given the high degree of genetic structuring among populations even at local scales (reviewed in De Meester et al., 2002). De Meester et al. (2002) presented a convincing synthesis that addressed this dispersal/gene flow problem. According to their "monopolization hypothesis", founder events, rapid population increase, local adaptation, resource monopolization, and the build-up of resting egg banks combine to make established populations highly resistant to invasion by new migrants. As such, patterns of genetic diversity

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are more related to priority effects than to contemporary dispersal opportunities. Genetic divergence among allopatric groups is often observed, but these groups most likely originated as a consequence of colonization priority rather than the presence of pervasive barriers to gene flow. Indeed, sharp demarcations can be observed in the distributions of refugial phylogroups, which appear to have dispersed rapidly until encountering habitats already occupied by other groups (Cox and Hebert, 2001; reviewed in De Meester et al., 2002). The importance of priority effects seems to be especially pronounced for cyclic parthenogens such as cladocerans and rotifers (Boileau et al., 1992; De Meester et al., 2002), which have the capacity for rapid local adaptation due to large population sizes, inter-clonal selection during parthenogenetic phases of reproduction, and sexual shuffling of genetic variation (De Meester et al., 2002).

Another important problem in the resolution of the dispersal/gene flow question is that of scale. Intuitively, it would seem that dispersal—followed by successful establishment—between continents would be even less likely than within them. As a result of differences in geology, climate, and habitat arrays, intercontinental migrants might be expected to have extra adaptive handicaps compared with incomers from the same continent, in addition to fewer candidate propagules that travel the distance. However, several species or closely related species pairs display close intercontinental genetic associations. Mitochondrial divergences of <5% are known between North and South America (Adamowicz et al., 2002, 2004; Hebert et al., 2003; Mergeay et al., 2008) and between North America and Europe (Schwenk et al., 2000; Ishida and Taylor, 2007) for several different species of *Daphnia*. Intercontinental associations are known within other cladoceran genera, such as *Bosmina* (Taylor et al., 2002), *Moina* (Petrusek et al., 2004), and *Holopedium* (Rowe et al., 2007), as well as in bryozoans (Freeland et al., 2000a) and rotifers (Fontaneto et al., 2008). The shallow divergences often observed are strongly suggestive of dispersal rather than ancient vicariance, if mitochondrial molecular clocks (e.g. Knowlton and Weigt, 1998) are correct even to within an order of magnitude. These successful intercontinental migrations suggest that additional factors might be fruitfully added to the monopolization hypothesis. For example, the degree of predator and pathogen adaptation to their local prey may be an important factor. Genetic evidence on presumed anthropogenic introductions indicates that intercontinental migrants can be more ecologically successful than would be expected based upon the numbers of newcomers alone (Havel et al., 2000; Mergeay et al., 2006). Understanding the dynamics of the dispersal/gene flow relationship across different spatial scales will require more complete knowledge of rates of intercontinental movement.

Daphnia is an especially useful target among freshwater zooplankton for a multi-continental study as a consequence of taxonomic insights gained from numerous genetic studies employing allozymes and mitochondrial DNA. These studies enable an interspecific phylogeographic analysis based upon genetically defined species boundaries, rather than on species lists derived only from morphological studies, which can overlook cryptic or recent speciation events (see Barraclough and Nee, 2001). Here, we quantify the frequency and phylogenetic depth of intercontinental shifts in *Daphnia*, compare patterns of divergence across different spatial scales, and assess the role of large-scale allopatric divergence in the global cladogenesis of this genus.

2. Methods

2.1. The sequence data

2.1.1. Species inclusion

All 92 *Daphnia* taxa included in this study are thought to be distinct species, even though some are not yet formally described. Undescribed species are referred to by the name of the species complex to which they belong, together with a number identifying the particular lineage and its region of origin (e.g. *D.* gr. *atkinsoni* sp2-ISR, referring to a previously unknown lineage in the *atkinsoni* complex from Israel). In some cases, genetic evidence has revealed that certain species are in fact divergent from a similar-looking morphospecies to which they have previously been assigned (e.g. North American lineages similar to *D. similis* or *D. pulex*). In these cases, where retaining information about the morphological similarity is useful, lineages differing from the nominate species are indicated by "cf." Species assigned invalid names or those which have not been formally described, but have received a name in previous literature (i.e. a *nomen nudum*), are designated by placing the specific epithet in quotation marks. Species names, localities, and abbreviations for regions, as well as taxonomic notes, are provided in Table 1.

Given this mixture of described and undescribed species, we briefly outline the species concept employed. Adamowicz and Purvis (2005) found that most authors of recent branchiopod genetic studies use a concordance species concept (Avise and Ball, 1990), or a proxy thereof (see below). Typically, a traditional morphospecies is employed as a starting point and then other types of evidence are considered to identify distinctive species with their own evolutionary trajectories. Here, a concordance species concept was also adopted, as it was expected that this approach would provide the best indication of species boundaries (i.e. where natural breaks lie in genetic, morphological, and ecological variation). We adopted a pragmatic set of quantitative criteria to delineate species following Adamowicz and Purvis (2005), who examined levels of genetic diversity typically observed within and among branchiopod species. Our preferred approach was to use multiple lines of concordant evidence when available, requiring that at least two of the following conditions be met: (1) a fixed allozyme allelic substitution at one or more loci; (2) overall divergence of allozyme allele frequencies; (3) mtDNA sequence divergence (>5% in sequences of the cytochrome *c* oxidase subunit I gene [COI], >4% in the 12S rRNA gene); (4) clear morphological difference; and (5) clear habitat difference or other ecological segregation. When multiple lines of evidence were unavailable, lineages showing substantial sequence divergence (>10% COI, >8% 12S) were treated as species, because in cases where there was more information, lineages differing by this magnitude were always classified as different species. However, *Daphnia "arenata"* Hebert (1995), did not conform to the above criteria, and was tentatively recognized as a distinct species because Pfrender et al. (2000) provided evidence of both a habitat shift and genetic differentiation from progenitor populations in allozyme, mtDNA, and microsatellite markers. This case may represent an example of incipient speciation. Two taxon names appearing in previous phylogenetic literature (*D. hyalina* and *D. thorata*) are not included here as they did not meet our species criteria; the former has been formally synonymized with *D. longispina* (for more details, see Petrusek et al., 2008).

The majority of the undescribed *Daphnia* species listed in Table 1, as well as many described species, have been characterized by allozymes (i.e. nuclear markers), which revealed that they possess fixed diagnostic differences (Hebert, 1977, 1995; Wolf and Mort, 1986; Hebert et al., 1989; Hobæk and Wolf, 1991; Taylor and Hebert, 1992, 1993a,b, 1994; Hebert and Finston, 1993, 1996, 1997; Dufresne and Hebert, 1994, 1997; Hebert and Wilson, 1994, 2000; Kořínek and Hebert, 1996; Taylor et al., 1996, 1998; Černý and Hebert, 1999; Kořínek et al., 2003; Michels et al., 2003; Adamowicz et al., 2004). In a few cases where allozyme data were lacking, the mtDNA divergence criterion was used instead. On the basis of mtDNA sequences, *D.* gr. *similis* sp2-EUR from Germany and *D.* cf. *"similis"* sp3-NA/SA from North America are very different from