

PREDISPOSED TO ADAPT? CLADE-LEVEL DIFFERENCES IN CHARACTERS AFFECTING SWIMMING PERFORMANCE IN DAMSELFLIES

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Abstract.—Previous studies have shown that two or three lineages of *Enallagma* damselflies, which historically co-existed with fish, recently invaded and adapted to living with large dragonfly predators in fishless waters. In adapting to live with these new predators, lineages shifted behaviorally to using swimming as an evasive tactic against attacking predators, evolved morphological features that made them faster swimmers, and evolved biochemical features to increase refueling strenuous activities like swimming. However, these habitat shifts have occurred in only one of the two primary clades within the genus in North America. Here, I show that clade-level differences exist among species in the ancestral, fish-lake habitat that should make habitat shifts easier to accomplish in the clade in which they have occurred. Specifically, fish-lake species in the clade in which habitat shifts occurred have much higher propensities to swim in the laboratory, swim faster when they do swim, and have higher mass-specific activities for arginine kinase than do species in the other primary clade, in which no extant species are found in fishless waters. These results are discussed in the context of the dynamics of founder events and the potential implications for community structure.

Key words.—Adaptation, damselflies, *Enallagma*, enzyme kinetics, Odonata, predator avoidance, swimming.

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All possible adaptations are not available to every taxon, because species do not acquire adaptations *ex nihilo*. Natural selection can only act on existing genetic and phenotypic variation within populations to favor more fit types (Loeschcke 1987; Falconer 1989; Hartl and Clark 1997). Consequently, many adaptations that would be extremely advantageous to a particular species are unattainable simply because of an ultimate lack of genetic variability. In addition, species are not completely free to explore all the possible adaptations within the area surrounding them in genotype and phenotype space. Natural selection will push populations and species uphill on the adaptive surface defined by the ecological environment until they reach a local fitness optimum (i.e., an adaptive peak: Wright 1931; Fisher 1958; Felsenstein 1979). Once there, it may be difficult to move to other nearby peaks (Wright 1932; Barton and Charlesworth 1984; Carson and Templeton 1984; Lande 1985, 1986; Whitlock et al. 1995).

These considerations indicate that the initial genotypic and phenotypic characteristics of a progenitor species can have tremendous influences on the types of adaptations that are potentially attainable and ultimately evolved by descendant lineages as they diversify. These problems may be particularly acute for lineages that are attempting to invade new ecological environments. For example, when a lineage of herbivorous insects shifts its diet to utilize a new host plant, the new host plant is usually similar in chemical composition to the ancestral host plants, and thus requires relatively small changes in the insect's phenotype to effectively utilize it (Ehrlich and Raven 1964; Farrell and Mitter 1993; Bercerra 1997). A lineage may be unable to invade a new environment requiring large phenotypic changes for adaptation either because the required changes are outside the possible scope of adaptation, given genetic variation and covariation in important traits, or founder populations may be continually driven extinct too quickly before they can adapt to the new ecological setting (Gomulkiewicz and Holt 1995). Consequently,

even sister clades may differ in the types of available adaptations and as a result the ranges of available ecological and evolutionary opportunities because of clade-level differences in phenotypes and genotypes; some would call such differences "phylogenetic constraints" (McKittrick 1993). This may explain why certain taxa flourish in a particular ecological environment while others are absent (Lawton 1984; Cornell 1985a,b; Cornell and Lawton 1992). A number of workers have identified phenotypic differences between sister clades that appear to significantly influence their relative rates of diversification (Mitter et al. 1988; Farrell et al. 1991; Barraclough et al. 1995; Hodges and Arnold 1995).

In this paper, I identify phenotypic differences between sister clades of damselflies that may explain why only one has invaded a new habitat. Species in the genus *Enallagma* (Odonata: Coenagrionidae) show a striking distribution pattern among the bogs, ponds, marshes, lakes, streams, and rivers of eastern North America; one group of species is found as larvae only in water bodies that also support fish populations, whereas the remaining species are found as larvae only in water bodies that lack fish (Johnson and Crowley 1980; McPeck 1989, 1990a, 1998; McPeck and Brown 2000). The results of field experiments have shown that predation by fish in lakes containing fish and predation by the larvae of large dragonflies in fishless waters are primarily responsible for enforcing this segregation of species between the two habitat types (I will hereafter refer to these as "fish lakes" and "dragonfly lakes," respectively, to emphasize the predator's importance in each system). *Enallagma* species in the two lake types are differentially vulnerable to these predators because they differ in overall levels of behavioral activity and in their responses to attacking predators; species in fish lakes are relatively inactive and rarely attempt to evade attacking predators, whereas species in dragonfly lakes are more active and swim away from attacking predators (Pierce et al. 1985; McPeck 1990b).

Phylogenetic analyses indicate that fish lakes are the an-

central habitat for the North American *Enallagma* and that at least two (and more likely three) independent invasions of the dragonfly-lake environment have occurred relatively recently (McPeck 1995a; McPeck et al. 1996; McPeck and Brown 2000; Brown et al. 2000). Recent phylogeographic and phylogenetic analyses confirm that these habitat shifts involved a lineage shifting from fish lakes into dragonfly lakes (M. A. McPeck, unpubl. data). Character reconstruction studies indicate that lineages shifting from fish lakes into dragonfly lakes rapidly evolved features that make them faster swimmers, presumably to augment the change to using swimming as an evasive tactic against attacking predators (McPeck et al. 1996; McPeck 1997). These lineages rapidly evolved larger caudal lamellae, the structures used to generate thrust during swimming (McPeck 1995a,b) and higher specific activity rates for at least one metabolic enzyme, arginine kinase, that influences the supply of energy to fuel swimming (McPeck 1999).

The deepest split in the phylogenetic hypothesis for the North American *Enallagma* divides the taxon into two groups with similar numbers of species in each clade (McPeck and Brown 2000; Brown et al. 2000). One clade containing 18 species has its center of diversity in New England, and the other primary clade containing 20 species has its center of diversity in the southeastern United States (for ease of presentation, I will refer to these two primary clades as the "northern" and "southern" clades, respectively; McPeck and Brown 2000). However, representative species in both clades can be found across North America (Westfall and May 1996; McPeck and Brown 2000). Of the 38 North American species, only four are dragonfly-lake species, and all four are members of the northern clade. The difference between clades in the frequencies of species in the two lake types is significant (Fisher's exact test, $P < 0.05$). This difference is not merely the result of biogeography, because two of the northern clade species are the only *Enallagma* in the dragonfly lakes of the southeastern United States; the range of one species extends into southern Georgia and Alabama, and the other has a range along the coastal plain of the southeastern United States from southern Mississippi, Alabama, and peninsular Florida north to Massachusetts (Westfall and May 1996; also see summary of biogeography in McPeck and Brown 2000).

In this paper, I identify phenotypic differences among species in these two clades that can account for why habitat shifts from fish lakes to dragonfly lakes have only occurred in the northern clade. In the laboratory, I measured the propensity of larvae to swim, swimming speed, and the mass-specific activity rates for arginine kinase, the enzyme that regulates the pool size of ATP, in 14 *Enallagma* species from across the phylogeny. These results show that the northern-clade species found today in fish lakes, the ancestral lake type, have a significantly higher propensity to swim, swim faster, and have a higher capacity to fuel activities such as swimming, than do southern-clade species. This may have made the habitat shift from fish to dragonfly lakes somewhat easier to accomplish for lineages in the northern clade.

MATERIALS AND METHODS

A total of 472 larvae of 14 *Enallagma* species were collected from the field during 1997 and 1998 from populations

in New Jersey, Massachusetts, New Hampshire, and Vermont. This set contains eight species from the northern clade, six from the southern clade, and at least two species from each of the major subclades within each of these primary clades (Fig. 1). Also, this set includes three of the four dragonfly-lake species in the genus. For most species, larvae from a number of widely separated populations were obtained, and for every species except *E. recurvatum*, larvae from very small instars through the final instar were included (for a detailed summary of body sizes and population locations for each species, see McPeck 1999). Total sample sizes for each species are given in Figure 1.

In the laboratory, each larva was first videotaped while swimming and then processed for enzyme analyses. Most larvae were swum and processed on the same day they were collected in the field, but some larvae had a gap of up to two days between collection and the swimming trial, depending on the distance between their natal lake and the laboratory in Hanover, New Hampshire. I found no effect of the time between collection and swimming trials on any variable in this study.

All swimming trials were performed in a 35.5 cm long \times 25.5 cm wide \times 6.0 cm deep Plexiglass tray filled with tap water and illuminated with indoor fluorescent lighting. Trials were videotaped using SuperVHS. A 30-cm clear ruler was attached to the bottom of the container for calibration during digitizing. All trials were performed at 20–22°C, which is well within the range of temperatures experienced by *Enallagma* larvae in the field. Larvae were held in the laboratory for at least 3 h at 20–22°C before being used in swimming trials. To achieve a large size range for each species, I collected larvae between April and December, and thus larvae collected on different dates had quite different thermal histories. After removing the effects of size on swimming speed and enzyme activity rates analyzed in this study, collection date was only significant at the $\alpha = 0.05$ significance level (uncorrected) in six of 56 comparisons, and none were significant when either Bonferroni or Dunn-Šidák corrections for multiple comparisons (Sokal and Rohlf 1995) were applied. Although thermal history of larvae may influence both their swimming performance and enzyme activities to some degree, any effects manifested in this study seem to be small relative to size and interspecific differences.

For each trial, one larva was gently released in the center of the container at the water surface. Each larva was coaxed to swim by gently tapping on its dorsal surface with a thin metal spatula. Each larva was prodded in this way until it performed four to six swims or clearly would not swim (i.e., gently tapping for 10–15 sec with no response). Many larvae of dragonfly-lake species would swim immediately following release in the container, but after this initial first swim, prodding with the spatula was always necessary to induce the remaining three to five swims. Fish-lake species would typically slowly sink to the bottom, orient themselves, and come to rest; I then prodded them to swim.

Videotapes were digitized using BioScan OPTIMAS® software (Optimas Corp., Bothell, WA). All methods of digitizing used here were the same as those described in McPeck et al. (1996), except that every fourth field was digitized instead of every third (filmed at 60 fields \cdot sec⁻¹). This was

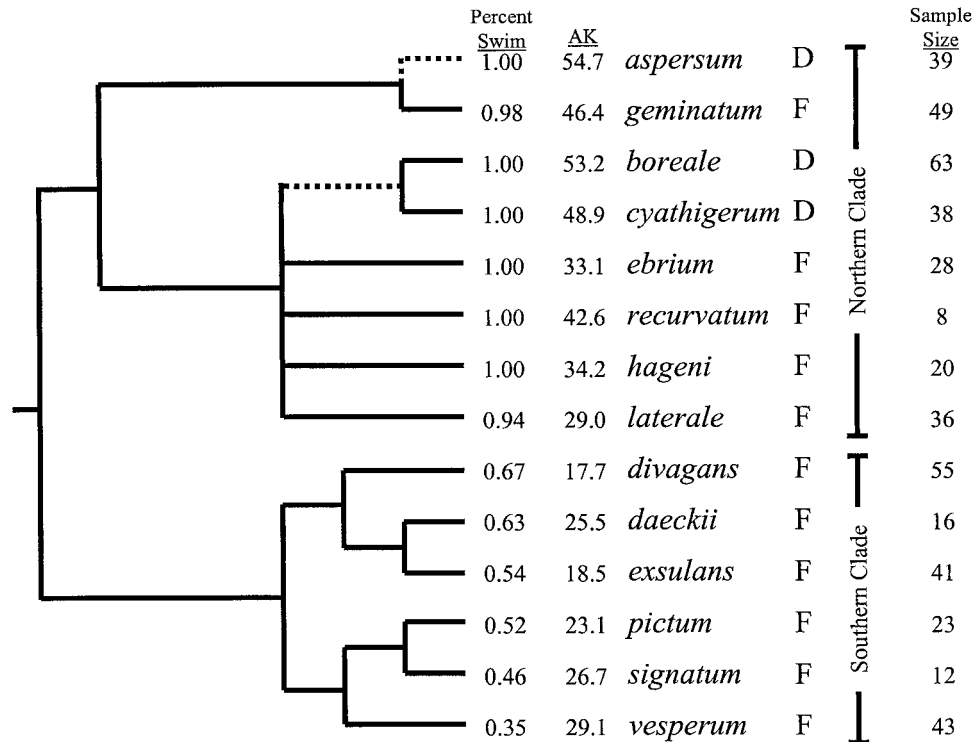


FIG. 1. Phylogenetic hypothesis for the 14 *Enallagma* species included in this study. The data and analyses supporting this tree are presented in Brown et al. (2000). Species are identified as to whether they are found as larvae in fish lakes (F) or dragonfly lakes (D). The two branches identified by dashed lines are those along which habitat shifts from fish lakes into dragonfly lakes are hypothesized to have occurred. The numbers at the tips of the branches are data for the percent of individuals that swam in laboratory trials, and least square means of the mass-specific activities of arginine kinase (AK in units of $\mu\text{M NADPH produced} \cdot [\text{g wet mass}]^{-1} \cdot \text{min}^{-1}$) taken from McPeck (1999). The sample size of each species included in this study are also given.

necessitated by changes to the software. From the data extracted from videotapes, swimming speed was calculated in units of $\text{cm} \cdot \text{sec}^{-1}$. A larva swims by rapidly moving its abdomen from side to side and using the caudal lamellae on the end of its abdomen to generate thrust. To quantify the intensity of this effort, the number of cycles of this beating during each swimming event was quantified and the rate of this beating was calculated by dividing this number by the duration of the swim (i.e., abdomen beat rate in units of $\text{beats} \cdot \text{sec}^{-1}$). Only data from the fastest swim for each individual is used in analyses presented in this paper.

For each species, I also calculated the percent of individuals that swam in trials. This variable ranged from 100% for some species to as low as 35% for others.

Within 2 h after the swimming trial, each larva was initially processed for enzyme analyses. The larva's three caudal lamellae were first removed for measurements of their size and shape (as in McPeck 1995a). In this paper, I present data on the lateral surface area of the median lamella; data on the lateral surface areas of the other two lamellae showed the same patterns as presented here. The exterior of the larva was blotted dry with a tissue and its wet mass was measured to the nearest 0.005 mg on an Ohaus Analytical Plus® balance (Ohaus Corp., Florham Park, NJ). The larva was then placed individually in an empty 1.5-ml microcentrifuge tube and frozen at -80°C for up to 8 months until enzyme analyses could be performed. I also found no effect of the time spent frozen on enzyme activity rates. The methods used to process

larvae and conduct the enzyme analyses have been presented elsewhere; see McPeck (1999) for details of these enzyme analyses. I assayed the activity of three enzymes: pyruvate kinase, lactate dehydrogenase, and arginine kinase. Only arginine kinase was found to have evolved higher rates of activity in lineages that underwent habitat shifts from fish lakes to dragonfly lakes (McPeck 1999). In that paper I showed that arginine-kinase-specific activity (in units of $\mu\text{M NADPH produced} \cdot [\text{g wet mass}]^{-1} \cdot \text{min}^{-1}$) decreased with increasing wet mass, but the slopes of relationships between arginine kinase activity and wet mass did not differ significantly among species (McPeck 1999). Therefore, I only analyze the results for arginine kinase in this paper. For this paper, I have calculated the least square means for each species from this previous analysis of covariance between arginine kinase specific activity and wet mass. Least square means are the predicted values of the response variable for each species calculated using the covariate relationship at the grand mean of the covariate for all species (Winer et al. 1991). This standardizes arginine kinase activities for comparisons among species to a common wet mass. I use these values to represent the average arginine kinase activity for the species.

I used nested analyses of covariance to test for differences between the northern and southern clades in swimming speed, abdomen beat rate, and lamella size. Each of these variables covary with body size intraspecifically, and so wet mass was used as a covariate to analyze them. Following previous analyses, species affiliations with the four major subclades in the

genus were included in the statistical model, along with species nested within clade as the error term for clade and wet mass as the covariate (see also McPeck 1995a; McPeck et al. 1996). The northern clade has two subclades, and the southern clade contains the other two subclades (Fig. 1). Because the northern-clade species are all very recent descendants from two species (i.e., the progenitors of the two subclades), this statistical structure maintains the natural taxonomic breaks between groups of species and thus represents the species-within-clade variance better than other possible breaks (e.g., having two groups—northern and southern clades only). Clade was assumed to be a fixed effect, and species nested within clade a random effect. Because of the imbalance in the number of species representing each of the four major subclades, the expected mean square for the species nested within clade term is slightly smaller than the correct error term for the clade effect. Care must therefore be taken when evaluating the significance of clade differences near $P < 0.05$. Linear contrasts were used to test for differences between the northern and southern clades, with species nested within clade used as the error term. All statistical analyses were performed using SAS (SAS Institute 1990).

RESULTS

Species-level differences in swimming propensity and enzyme activities were clearly evident between the northern and southern clades (Fig. 1). Species in the northern clade had significantly higher arginine kinase activities than southern-clade species ($t_{12} = 4.48$, $P < 0.001$). Previous analyses showed that arginine kinase activities were greatly increased in association with habitat shifts from fish to dragonfly lakes (McPeck 1999). Therefore, the clade-level differences for average arginine kinase activity could be primarily due to adaptation associated with habitat shifts and not representative of clade differences as a whole. This is not the case; the clades still differ in arginine kinase activities when only fish-lake *Enallagma* are included in the analysis ($t_9 = 3.81$, $P < 0.005$).

Northern-clade species also had substantially higher propensities to swim than southern-clade species (Fig. 1: percent of individuals that swam when only fish-lake species are included in the analysis, $t_{5.5} = 9.50$, $P < 0.0001$; degrees of freedom are adjusted to correct for unequal variances between the two groups). Overall, only three individuals of the northern-clade species did not swim in the laboratory. In contrast, between one-third and two-thirds of individuals in southern-clade species did not swim, depending on species (Fig. 1).

In addition to higher propensities to swim, larvae of species in the northern clade also swim faster than those of southern-clade species that would swim (Fig. 2b). As would be expected given the large numbers of species and individuals included in this study, significant heterogeneity in slopes was detected among species for swimming speed regressed on wet mass; this was true whether dragonfly-lake species were included ($F_{13,354} = 4.27$, $P < 0.0001$) or excluded from the analysis ($F_{10,220} = 2.24$, $P < 0.02$). The slopes were qualitatively similar, and the correlation between the least square species means estimated from an analysis in which slopes were allowed to vary among species and an analysis

of covariance that constrains all species to have the same slopes were highly correlated ($r_{10} = 0.95$, $P < 0.0001$ when only fish-lake species are included). Therefore, I do not believe that the heterogeneity in slopes among species is sufficiently large to affect the conclusions that can be drawn from the analysis. On average, fish-lake species in the northern clade swam significantly faster than species in the southern clade (only fish-lake species included in the analysis: $F_{1,6} = 22.57$, $P < 0.002$). In addition, dragonfly-lake species swam significantly faster than their fish-lake counterparts in the northern clade ($F_{1,7} = 8.10$, $P < 0.03$). (There are two notes about this test. First, it is conservative because the error mean square for this particular test is larger than the correct error mean square for the test; second, an explicit test including all fish-lake species from both clades in this nested analysis is not possible, but the fact that dragonfly-lake species swim significantly faster than fish-lake species from the southern clade is evident by deduction.) When the least-square means of the regression lines are compared, the dragonfly-lake species swam on average 42% faster than the fish-lake species in the northern clade and the fish-lake species in the northern clade swam on average 49% faster than the fish-lake species in the southern clade (Fig. 2a, b).

The differences in swimming speed between the larvae of fish-lake species in the northern and southern clades were primarily the result of behavioral differences. Previous analyses have shown that larvae swim faster if they have larger lamellae and if they beat their abdomens faster (McPeck et al. 1996). As with the analysis of speed, the slopes of regressions for both lamella size and abdomen beat rate were also both significantly heterogeneous among species (Fig. 2c–f: lamella area: $F_{13,354} = 8.58$, $P < 0.0001$; abdominal beat rate: $F_{13,354} = 6.92$, $P < 0.0001$). I analyzed these two variables in similar fashion to swimming speed, again because the correlations between least square means assuming and not assuming equal slopes were similar (both with correlations of $r_{10} > 0.93$, $P < 0.0001$).

Larvae of fish-lake species from the northern clade beat their abdomens faster than those from southern-clade species ($F_{1,7} = 79.49$, $P < 0.0001$). However, over the entire size range of larvae, southern-clade species tended to have larger lamellae for a given wet mass than northern-clade, fish-lake species ($F_{1,7} = 5.85$, $P < 0.05$; but recall that the significance of this test is slightly inflated by an imprecise error term, and so this test must be interpreted with great caution). The slope for the entire group of fish-lake species from the southern clade was significantly steeper than that for the northern-clade group (Fig. 2d: $F_{1,238} = 4.02$, $P < 0.05$). The smallest larvae in both groups had similar-sized lamellae, but the ontogenetic relationship between lamella size and wet mass increases faster for the southern clade (Fig. 2d). Consequently, at larger sizes, larvae from fish lakes in the southern clade had larger lamellae than fish-lake species in the northern clade. (Note that dragonfly-lake species had larger lamellae than their fish-lake counterparts in the northern clade [$F_{1,6} = 13.93$, $P < 0.01$]. This is identical to the result found for lamella size when only final instars of species were compared using data from other larvae [McPeck 1995a].) Thus, larvae from fish-lake species in the northern clade swim faster than fish-lake species in the southern clade because they beat their

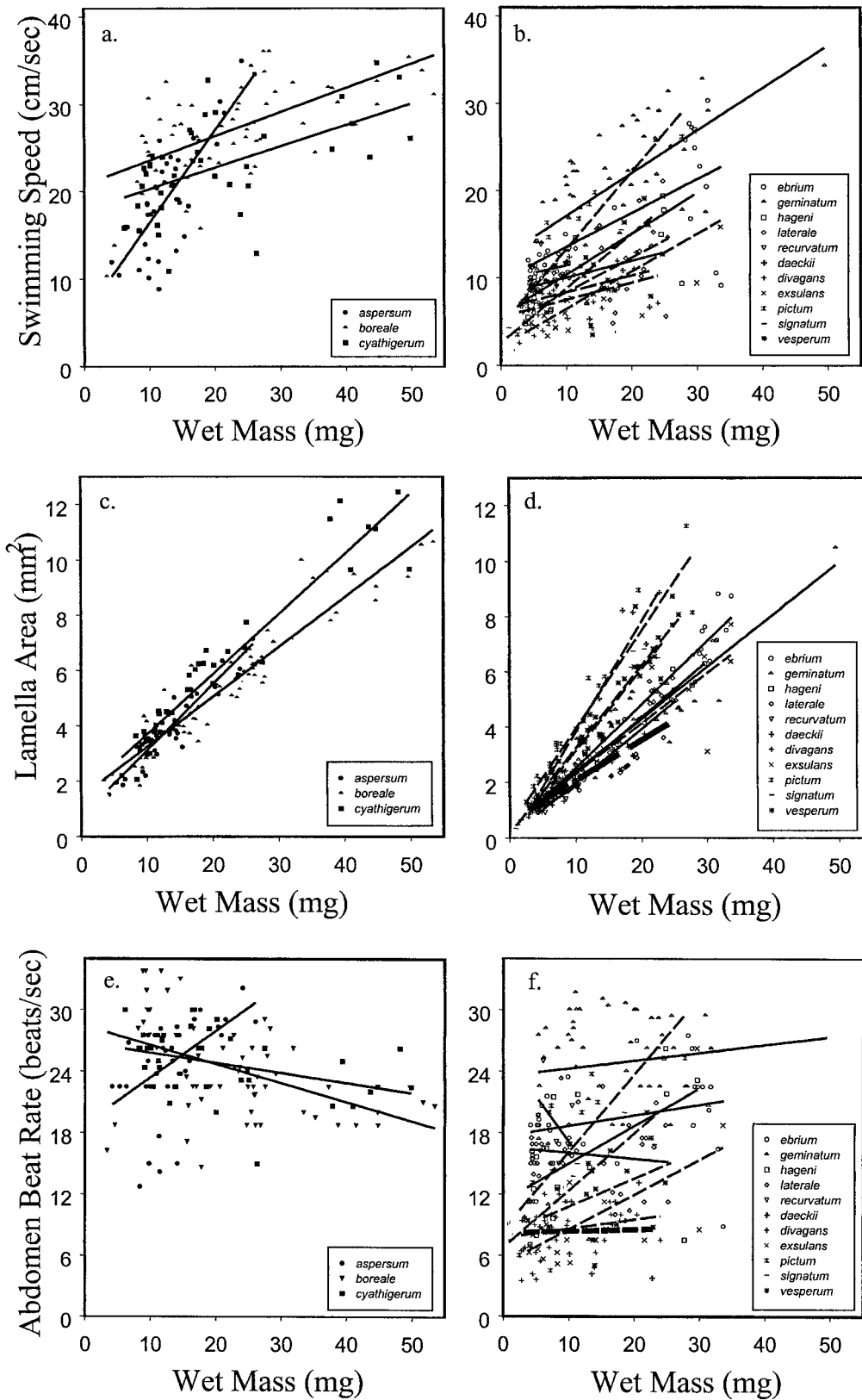


FIG. 2. Swimming variables measured on larvae of 14 *Enallagma* species. The variables are swimming speed ($\text{cm}\cdot\text{sec}^{-1}$) for (a) dragonfly-lake and (b) fish-lake species; lateral surface area of the median lamella (mm^2) for (c) dragonfly-lake and (d) fish-lake species; and the abdomen beat rate ($\text{beats}\cdot\text{sec}^{-1}$) for (e) dragonfly-lake and (f) fish-lake species. Each symbol is the value for a single larva. The regression lines for species in the northern clade are solid and those for the southern-clade species are dashed.

abdomens faster and not because they have larger caudal lamellae (Fig. 2).

DISCUSSION

Swimming away from threatening predators was a key evolutionary adaptation that allowed lineages of *Enallagma* to invade fishless waters in eastern North America and coexist with large, active dragonfly larvae (McPeck et al. 1996; McPeck 1997; McPeck and Brown 2000). In the ancestral fish-lake environment, selection operates against swimming as an evasion tactic, because damselfly larvae have little chance of outswimming a fish. Fish-lake *Enallagma* avoid fish predators primarily by crypsis (Pierce et al. 1985; McPeck 1990b). In contrast, dragonfly predation continues to impose substantial phenotypic selection for morphological features that enhance swimming speed in dragonfly lakes (McPeck 1997) and presumably for other physiological and behavioral features that enhance swimming propensity and speed. Lineages shifted behaviorally from not evading attacking predators to using swimming as an evasive tactic when they adapted to living with dragonfly predators (Pierce et al. 1985; McPeck 1990b). Also, these lineages evolved increased swimming speed by evolving increases in the sizes of their caudal lamellae to increase the thrust that can be generated during swimming (McPeck 1995a,b), and they evolved changes in molecular physiology to sustain the metabolic work of swimming by evolving higher mass-specific arginine kinase activities (McPeck 1999). In addition to these differences affecting swimming speed, the dragonfly-lake lineages also apparently evolved higher overall activity levels (e.g., increased frequency and rates of movement, increased rates of food search) as compared to their fish-lake counterparts (Pierce et al. 1985; McPeck 1990b).

When a lineage invades a new ecological environment, individuals in the founder population will usually be maladapted to the ecological conditions found there. Initially, the new environment will probably be a sink, where demographic conditions cause population decline and so the population can be maintained only by continual immigration (Pulliam 1988). If founding a population in this new environment is a rare event and subsequent immigration is also rare, the typical fate of such a founder population will be extinction because of individuals' maladaptation. Such a founder population will be successful in the long run only if it can quickly and sufficiently adapt to its new ecological surroundings to turn this environment from a sink into a source, where demographic conditions promote positive population size even in the absence of immigration (Gomulkiewicz and Holt 1995). The time required for this adaptation will depend on both the phenotypic distance that must be crossed and the genetic architecture of ecologically important characters (i.e., heritabilities and genetic correlations). Thus, a founding population is in an evolutionary race to adapt to the new selective environment before being driven extinct, and the more maladapted the founding population begins, the less likely it is to adapt sufficiently before extinction occurs (Gomulkiewicz and Holt 1995). In phenotypic terms, this idea can be restated as follows: The farther the founder population begins away from the new optimal phenotype (i.e., the farther

from an adaptive peak), the less likely it is to adapt sufficiently before being driven extinct.

The results of this study indicate that founder populations of lineages in the northern clade would begin much closer to the multivariate phenotype favored by dragonfly predation, at least in three important characters, than would founder populations from southern-clade species. First, northern-clade species have an innately higher propensity to swim than do southern-clade species. Although fish-lake species rarely swim from attacking predators (Pierce et al. 1985; McPeck 1990b), larvae of northern-clade species are easily coaxed to swim in the laboratory, whereas one-third to two-thirds of larvae from southern-clade species refuse to swim under the same conditions (Fig. 1). I interpret this behavior in the laboratory as indicative of latent species differences between the two clades in their propensities to swim.

Larvae of fish-lake species in the northern clade also swim significantly faster on average than larvae of southern-clade species (Fig. 2b). This difference is primarily due to their having greater rates of abdominal beating while swimming. Fish-lake species from the southern clade appear rather lethargic when swimming, slowly waving back and forth. In contrast, fish-lake species from the northern clade wave their abdomens from side to side while swimming, with almost the same intensity as dragonfly-lake species (Fig. 2f).

Finally, the two clades differ in the specific activities of at least one enzyme that is important to fueling metabolic activities like swimming. Arginine kinase is the enzyme in most invertebrates that acts to regulate ATP pool size by maintaining a high ATP:ADP ratio (Blethan and Kaplan 1968; Morrison 1973). During the first few seconds of strenuous metabolic work, arginine kinase is the enzyme that initially replenishes ATP as it is depleted (Schneider et al. 1989; Wegener et al. 1991; for a review of ATP turnover in vertebrate muscle, see Hochachka and Matheson 1992). Previous analyses have shown that the rapid evolution of higher arginine kinase mass-specific activities were also associated with habitat shifts from fish lakes to dragonfly lakes (McPeck 1999). Presumably, natural selection favored increases in arginine kinase mass-specific rates to increase the rate at which ATP is replenished and thus to maintain an adequate amount of ATP during the few seconds of maximal exertion required when swimming away from an attacking dragonfly and to recharge the ATP pool rapidly following a predator escape (McPeck 1999).

Greater latent swimming propensities, faster swimming speeds, and higher arginine kinase activities would all have made adapting to dragonfly predation in dragonfly lakes quicker and easier for fish-lake species in the northern clade as compared to southern clade. For all these characters, founder populations of fish-lake species in the northern clade would begin closer to what is favored by natural selection in the dragonfly-lake environment, as compared to southern-clade species. Obviously, adapting to this new ecological milieu was and remains extremely difficult for even northern-clade species, given that only two or three habitat shifts have been successful within the last 15,000 years (McPeck and Brown 2000), in spite of the fact that founder populations of fish-lake species in both clades are continually being established in dragonfly lakes each year (McPeck 1989, pers. obs.).

However, the difference in initial phenotypes may be enough to ensure that southern-clade founder populations are always eventually driven extinct before they can adapt, while at least a few northern-clade founders have been successful.

Such differences do not have to be apparent in all characters that would provide advantages in the new environment for successful habitat shifts to occur. In *Enallagma*, one of the critical adaptive changes that occurred when lineages shifted into dragonfly lakes was an increase in lamella size to increase the thrust per stroke during swimming (McPeck 1995, 1997; McPeck et al. 1996). In contrast to these other characters, fish-lake species in the northern clade did not begin with an advantage in lamella size. Lamella size was clearly malleable to natural selection imposed by dragonfly predation (McPeck 1997), but lamella size did not favorably bias the chances of a habitat shift in the northern clade.

In addition to the phenotypic distance over which a founder population must adapt, two general features of a system could influence the rate at which evolutionary habitat shifts occur (as identified in the North American *Enallagma* clade): (1) the frequency and interspersedness of the various habitat types; and (2) the rate at which founder populations are established in the novel habitat type. At present, assessment of the relative frequencies of fish- and dragonfly-lake habitats across North America is difficult, because the widespread introductions of fish into previously fishless waters over the last century have probably greatly shifted the balance toward waters containing fish and have obscured patterns that existed before Europeans colonized North America (e.g., Knapp and Matthews 1998). However, water bodies supporting *Enallagma* species that have and lack fish remain interspersed across the landscape on a local scale, with substantial opportunities for dispersal between them (Johnson and Crowley 1980; McPeck 1989, 1990a, 1998). Dispersal between water bodies that are less than 1 km apart is very low for most *Enallagma* species (McPeck 1989; Anholt 1990), but females of fish-lake species in both clades do sometimes oviposit in dragonfly lakes (McPeck 1989, pers. obs.). Because *Enallagma* adults are apparently incapable of discriminating between fish lakes and dragonfly lakes (McPeck 1989), habitat shifts are most likely the result of females simply ovipositing in the wrong lake and not the result of changes in active habitat choice.

The lack of successful habitat shifts in the southern clade also cannot be attributed to biogeographic limitations. Presently, northern-clade species inhabit dragonfly lakes across much of North America (Westfall and May 1996; McPeck and Brown 2000). Overlaying the ranges of the four dragonfly-lake species suggests that *Enallagma* species are absent in dragonfly lakes only in the area from Nebraska and eastern Colorado south through Texas, then east through Louisiana to central Mississippi, and west from southern New Mexico and Arizona northwest into central Nevada. At least one dragonfly-lake *Enallagma* species can be found in all other parts of North America, including all of the southeastern United States (Westfall and May 1996; McPeck and Brown 2000). Thus, ecological opportunities must have been available for habitat shifts to have occurred in the southeastern United States by lineages in the southern clade.

Also, several lines of evidence suggest that it is unlikely that the northern-clade species have displaced southern-clade

species from dragonfly lakes in the southeastern United States. First, the results of field experiments have identified no asymmetrical competitive effects for damselfly species within either lake type (Johnson et al. 1984; McPeck 1990a, 1998). Second, species that have resulted from the two or three habitat shifts that have occurred in the northern clade have all converged onto very similar phenotypes (Pierce et al. 1985; McPeck 1990b, 1995a,b, 1999; McPeck et al. 1996; McPeck and Brown 2000). As a result, all *Enallagma* species within each lake type are ecologically very similar (McPeck 1990a, 1998; McPeck and Brown 2000). Also, species in other genera of the Coenagrionidae that are also found only in dragonfly lakes (e.g., *Coenagrion*) share many of the larval phenotypic features of the dragonfly-lake *Enallagma* and co-occur with them (Walker 1953; Westfall and May 1996; M. A. McPeck, pers. obs.). Finally, northern-clade and southern-clade species can be found living in the same fish lakes across North America, with some areas of the continent having up to 12 species in the same lakes depending on the local species pools (Johnson and Crowley 1980; McPeck 1990a, 1998; McPeck and Brown 2000). Given these ecological and biogeographic facts, it is difficult to imagine how northern clade species would have displaced southern clade species in dragonfly lakes.

Differences in the potentials for members of various clades to invade new ecological milieus will also potentially influence the types and structures of biological communities that develop over evolutionary time in a given region. Studies of herbivorous terrestrial insects have provided some of the best illustrations. For example, Cornell (1985a,b) found very different assemblages of cynipid gall wasps feeding on seemingly similar species of oaks in California. He attributed these differences in herbivore assemblages to the capabilities of the insects to colonize and adapt over evolutionary time to the different oaks, although the specific capabilities were unknown (Cornell 1993). Also, Lawton (1984) has found huge differences in the number and types of insects feeding on bracken ferns (*Pteridium aquilinum*) around the world. He has attributed these differences to variation in the regional pools of insect herbivores available to utilize bracken ferns. Cadle and Green (1993) have also shown that the ecological niches filled by snakes in a given region depend on the snake clades that occupy that region. If only the southern clade of *Enallagma* existed in North America (e.g., if the northern clade had been completely wiped out during the glaciation that apparently triggered its recent radiation; McPeck and Brown 2000), *Enallagma* lineages may never have successfully invaded dragonfly ponds and lakes, and thus would never have filled the niche that they occupy today in dragonfly lakes. Consequently, the dynamics of the littoral food web in dragonfly lakes could now be very different from what would have been if *Enallagma* had never invaded this habitat (e.g., for relevant food web dynamics, see models in Holt et al. 1994; Leibold 1996; McPeck 1996). How any system would change with the invasion of a new taxon will depend on the particular dynamic features of that system and the performance capabilities of the invader. The likelihood of such change will depend on the existence of taxa with opportunities to invade and with the evolutionary capabilities to adapt successfully to their new surroundings.

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