

## PARALLEL EVOLUTION IN ECOLOGICAL AND REPRODUCTIVE TRAITS TO PRODUCE CRYPTIC DAMSELFLY SPECIES ACROSS THE HOLARCTIC

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**Abstract.**—The damselfly genus *Enallagma* originated in the Nearctic, and two Nearctic lineages recently underwent radiations partly associated with multiple independent habitat shifts from lakes dominated by fish predators into lakes dominated by dragonfly predators. A previous molecular study of four Palearctic morphospecies and all representative Nearctic species identified the presence of two cryptic species sets, with each set having Palearctic and Nearctic representatives. However, the cryptic species within each set are not sibling species. Here, we present quantitative data on ecologically important larval morphologies and behaviors involved in predator avoidance and on adult male morphological structures involved in mate recognition to quantify the phenotypic relationships among these cryptic species sets. For the adult stage, our data indicate strong parallel evolution of the structures involved in specific mate recognition—the male cerci. For the larval stage, morphometric analyses show that the Palearctic species evolved a nearly identical morphology to the sibling-clade members in the Nearctic that live in waters where dragonflies are the top predators. This implicates the importance of dragonfly predation in the history of the Palearctic clade. Behavioral analyses suggest population differentiation in response to the actual predator environment in the Palearctic clade, consistent with the species differentiation seen in the Nearctic. Our results suggest parallel evolution of adult traits that influence specific mate choice and larval traits that influence ecological performance underlie the striking similarity of *Enallagma* species across continents. This concurrent parallel evolution in both stages of a complex life cycle, especially when both stages do not share the same selective environment, may be a very unusual mechanism generating cryptic species.

**Key words.**—Antipredator behavior, cryptic species, ecomorphology, habitat shifts, parallel evolution, specific mate recognition.

Received April 6, 2005. Accepted June 30, 2005.

Natural selection is a fundamental source of ecological and reproductive differentiation ultimately leading to speciation and the generation of biodiversity (Schluter 2000, 2001; Eberhard 2001; Gavrillets 2003). The responses of different lineages that experience the same selective regime in different locations frequently lead to the evolution of similar phenotypic solutions, a process known as parallel evolution (Fitch 2000). For example, *Anolis* lizards have repeatedly diversified into the same suite of niches on islands throughout the Caribbean (Losos et al. 1998). Likewise, sticklebacks have diversified into limnetic and benthic forms in multiple lakes (Schluter and Nagel 1995; Albert and Schluter 2004; Boughman et al. 2005). Parallel evolution is expected when different lineages experience similar ecological conditions, such as those associated with microhabitat use and diet, since natural selection will often favor similar solutions to the ecological problems faced by lineages in different geographic areas (e.g., Schluter and Nagel 1995; Losos et al. 1998; Rüber et al. 1999; Cassens et al. 2000; but see Leal et al. 2002).

Although sexual selection is thought to be much less predictable in outcome than natural selection, convergent phenotypes involved in mate recognition may arise in allopatric lineages as a result of sexual selection and the evolution of specific mate recognition traits (e.g., Henry et al. 1999; Kopp and True 2002; Allender et al. 2003). In most cases, the different species having convergent mating phenotypes can still be distinguished based on other traits. In the extreme,

however, evolving similar mate recognition traits can result in cryptic species, which can only be recognized as distinct species by molecular studies (e.g., Henry et al. 1999; Kopp and True 2002; Allender et al. 2003). Such parallelism is typically attributed to genetic and ecological constraints (Eberhard 2001; Orr 2001; Allender et al. 2003).

Most animals have a complex life cycle with morphologically distinct stages, typically a larval and an adult stage (Buss 1987; Werner 1988). Because selection pressures may differ greatly among different life stages, particularly when life stages occupy different habitats (Moran 1994), concurrent parallelism of both stages may be merely a matter of chance. This may be especially true when sexual selection causes parallel evolution in the adult stage, although certain forms of sexual selection (e.g., sensory biases) may favor similar phenotypes evolving in different lineages. However, a strong genetic linkage between stages could also indirectly cause similar trajectories of evolution in both stages. Many cases of parallel and convergent evolution have focused on only one life-history stage, so few data exist to evaluate whether such parallelism in both stages occurs. Although parallel evolution in only one life stage is much more common, one recent example identified parallel convergence in larval and adult traits in the morphologies of ranid frogs of Madagascar and Asia (Bossuyt and Milinkovitch 2000).

In this paper, we explore phenotypic diversification in the larval and adult stages among two groups of cryptic *Enallagma* damselfly species (Odonata: Coenagrionidae) in a clade that radiated across the Holarctic (Fig. 1). The ecological and reproductive processes known to affect phenotypic

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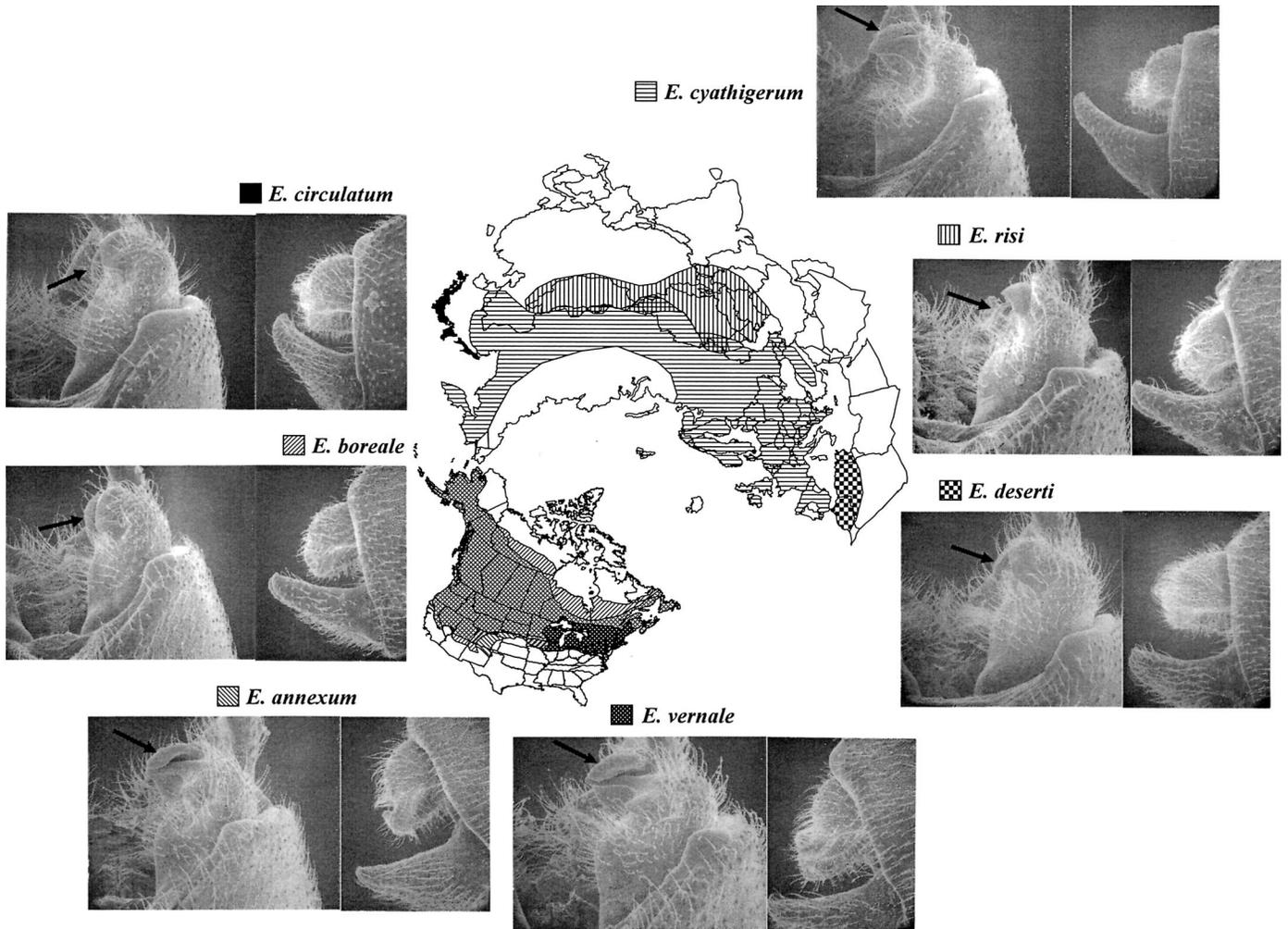


FIG. 1. Distributions of the three Nearctic and the four Palearctic *Enallagma* cryptic species with the *boreale*-type and *cyathigerum*-type cerci. The distribution of *E. vernale* is completely included in the ones of the Nearctic *E. boreale* and *E. annexum*. For each species a dorsal (left panel) and lateral (right panel) view of the cercus is presented. The pale tubercle is indicated by an arrow for each species. Note the distal position of this tubercle in Palearctic *E. cyathigerum* and Nearctic *E. annexum* and *E. vernale*, which causes the upcurved ventral apex in lateral view. Also, note the medioventral position of this tubercle in the rest of the species, which causes the lack of the upcurved apex in lateral view.

diversification in the genus are well understood (see Study System). We present and integrate data on ecologically important morphologies and behaviors involved in larval predator avoidance and on adult morphology involved in specific mate recognition. These analyses, together with previous work, show striking parallelism of taxa across continents in traits that influence larval ecology and adult mate choice. We consider the selection pressures that shaped differences in larval morphology and behaviors among the Nearctic species to interpret similarities and differences between Palearctic and Nearctic species as reflecting evolutionary responses to the similarities and differences in their respective predation regimes. Also, by combining this with information on larval ecology, we infer the processes driving the evolutionary and ecological diversification within the genus *Enallagma* across the Holarctic and suggest testable hypotheses for how the structure and organization of littoral food webs across the Holarctic may vary with the fish faunas.

#### Study System

Four Palearctic and three Nearctic *Enallagma* species form two highly cryptic species sets that span the Holarctic. The taxonomic status of these seven species has been long debated (e.g., Donnelly 1989; May 1997; Samraoui et al. 2002; Kosterin 2004). *Enallagma* species are most easily and consistently recognized by morphological features of the superior caudal appendages of males (i.e., the cerci; Westfall and May 1996). *Enallagma* males use their cerci to grasp females during mating, and females use the tactile cues they receive from these structures to determine the suitability of a male for mating (Paulson 1974; Robertson and Paterson 1982). Based on cercus morphology, some authors (Jurzitza 1975; May 1997) have suggested that the seven focal species of this study could be classified into two Holarctic taxa: (1) the Nearctic *E. annexum* and *E. vernale* and the Palearctic *E. cyathigerum*; and (2) the Nearctic *E. boreale* and the Pale-

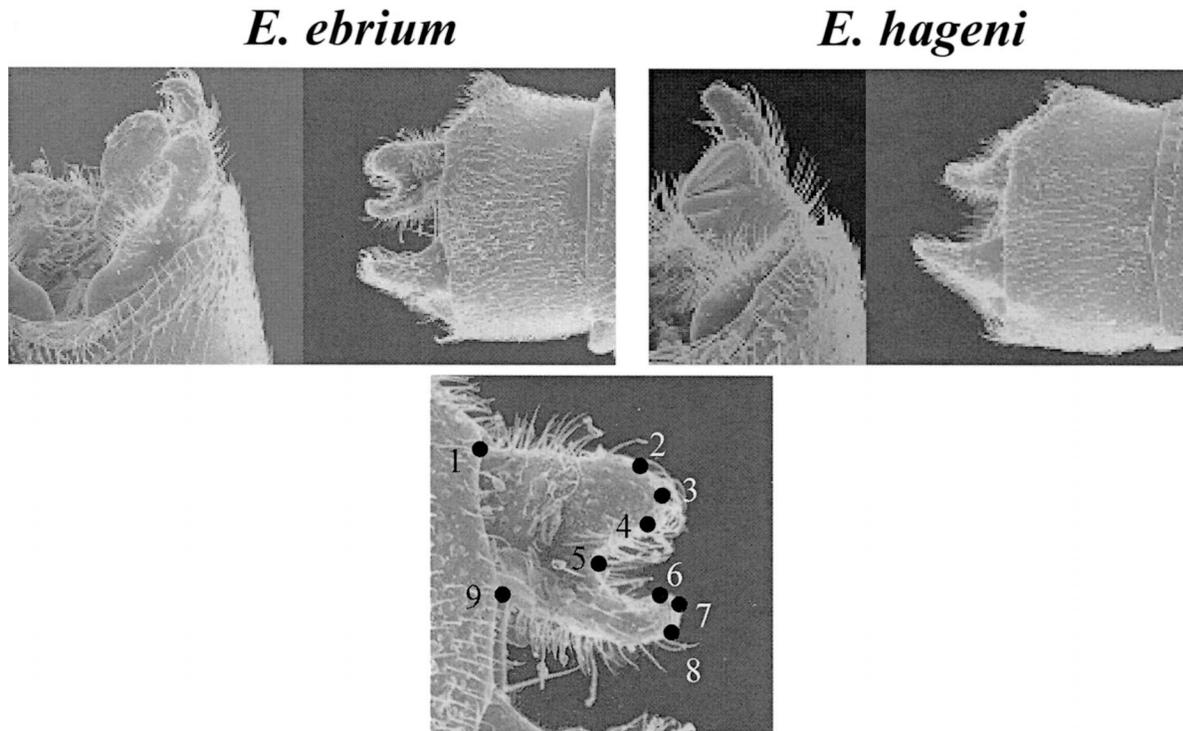


FIG. 2. Dorsal and lateral views of the cerci for *Enallagma ebrium* and *E. hageni*, the species inferred by Turgeon et al. (2005) as the two possibilities for the progenitor of the radiation that produced the Nearctic members of the cryptic species complex illustrated in Figure 1. In the lateral views, the 10th abdominal segment is shown. The cercus is the superior appendage, and the paraproct is the inferior appendage. The bottom panel shows the lateral view of an *E. ebrium* cercus. Black dots identify the nine landmarks used in the morphometrics analysis. Each distance used in the analysis is identified here by the number of the two landmarks in brackets: distance 1 [1,2], distance 2 [1,3], distance 3 [1,4], distance 4 [1,7], distance 5 [2,3], distance 6 [3,4], distance 7 [2,4], distance 8 [3,5], distance 9 [5,7], distance 10 [6,7], distance 11 [7,8], distance 12 [6,8], distance 13 [3,9], distance 14 [6,9], distance 15 [7,9], distance 16 [8,9], distance 17 [3,7], distance 18 [1,9], distance 19 [1,5], distance 20 [5,9].

arctic *E. circulatum* (Japan), *E. risi* (Central Asia), and *E. deserti* (North Africa). These two groups show one of two cercus types (Fig. 1). The first group has the *cyathigerum*-type cercus, with a pale tubercle distal to a robust terminal tooth that forms the upturned apex as seen in lateral view (Fig. 1). In contrast, the second group has the *boreale*-type cercus, with a pale tubercle proximal and medioventral to the terminal tooth, which creates only a short ventral extension of the apex in lateral view (Westfall and May 1996; Kosterin 2004; Fig. 1). These seven species are also strikingly similar in overall larval and adult morphology (Westfall and May 1996).

In contrast to the general morphological groupings, a recent molecular study showed strong segregation of these species into separate Nearctic and Palearctic clades. Analyses of mitochondrial DNA (mtDNA) sequences place the Palearctic species in a clade that is sister to the clade containing the Nearctic species, and these two clades were estimated to have diverged 1.0–1.3 million years ago (Turgeon et al. 2005). Analyses using amplified fragment length polymorphism markers also show that the Nearctic and Palearctic species form distinct clades (Turgeon et al. 2005). Thus, each group of cryptic morphological species is in fact composed of Nearctic and Palearctic species that have diverged some time ago. Turgeon et al. (2005) inferred that either *E. hageni* or

*E. ebrium* (both species with very different cercus types [Fig. 2] and very different larval morphologies) was the progenitor of the Nearctic radiation from which *E. annexum*, *E. boreale* and *E. vernale* were derived. This inference was based on two pieces of data: (1) these two species show the greatest level of sequence variability in the radiation; and (2) they both have haplotypes that span a fragmentation event in the haplotype network for the radiation, while the remaining species from the radiation do not. Thus, any phenotypic similarity between Nearctic and Palearctic species within each group is not the result of their being directly descended from a common ancestor that had the same phenotypes they now possess. Rather, the similarities between these groups of cryptic species must have resulted from strong parallel evolution in both adult cerci and overall larval morphologies.

Because the Palearctic clade is embedded in a much larger Nearctic clade, Palearctic *Enallagma* appear to have originated from an invasion of the Palearctic by a Nearctic lineage (Turgeon et al. 2005). This larger clade contains 38 Nearctic species, with its greatest diversity in eastern North America (Westfall and May 1996; Brown et al. 2000; McPeck and Brown 2000). In eastern North America, species segregate ecologically into two disjunct groups based on larval habitat. Most species are found as larvae only in water bodies where fish are the top predators (hereafter referred to as “fish

lakes''; Johnson and Crowley 1980; Pierce et al. 1985; McPeck 1990a, 1998; McPeck and Brown 2000). In contrast, four species, including *E. annexum* and *E. boreale*, are found as larvae only in fishless waters where large, active dragonfly species are the top predators (hereafter referred to as ''dragonfly lakes''; Johnson and Crowley 1980; McPeck 1990a, 1998). *Enallagma vernale*, the other cryptic Nearctic species, is found with fish.

Nearctic *Enallagma* species segregate between these two predation regimes because they differ in ways that make them differentially susceptible to fish and dragonfly predation (Pierce et al. 1985; Blois-Heulin et al. 1990; McPeck 1990a,b; McPeck et al. 1996). Phylogenetic reconstructions have shown that fish lakes are the ancestral larval habitat for the Nearctic *Enallagma*, and the four Nearctic dragonfly-lake species are the result of three independent adaptive habitat shifts that occurred within the last 110,000 years (Brown et al. 2000; McPeck and Brown 2000; Turgeon et al. 2005).

Functional phenotypic studies have also identified critical phenotypes involved in these habitat shifts. Fish-lake species are relatively inactive even in the absence of predators, they respond to the presence of both fish and dragonflies by decreasing their activity, and they generally do not try to evade attacking predators (Pierce et al. 1985; McPeck 1990b, 2000; McPeck et al. 1996; Stoks et al. 2003). In contrast, dragonfly-lake species are active when predators are not nearby, they decrease their activity in the presence of dragonflies but not fish, and they swim away from attacking predators (Pierce et al. 1985; McPeck 1990b, 2000; McPeck et al. 1996; Stoks et al. 2003), which is an effective evasive tactic against dragonflies but disastrous against fish (Pierce et al. 1985; Stoks and De Block 2000). In addition, dragonfly-lake species are also adapted morphologically and biochemically to make them much faster swimmers than fish-lake species: they have larger caudal lamellae to generate thrust and they have wider abdomens, presumably as a result of greater muscle mass to generate more power for thrust (McPeck 1995a,b, 1997, 1998, 2000; McPeck et al. 1996).

The ecological pattern of larval segregation appears to be only applicable to the eastern half of North America, where the fish fauna has been historically dominated by centrarchid fishes (Page and Burr 1991). Outside this area and in high-altitude ponds and lakes in the east, the fish fauna is dominated by relatively ineffective littoral predators in the Salmonidae and Cyprinidae, and *Enallagma* species do not segregate between fish-containing and fishless waters (Anholt 1990; Chivers et al. 1996; M. A. McPeck, unpubl. data; D. Paulson, pers. comm.). Our experience in eastern North America indicates that these lakes typically support low to moderate densities of the large dragonfly species (e.g., *Anax*, *Aeshna*, and *Tramea* species) that characterize truly fishless waters. Likewise, disjunct larval habitat affinities are not found in the Palearctic, where these same fish groups dominate (Corbet 1999; Samraoui et al. 2002; R. Stoks, pers. obs.; O. Kosterin, pers. comm.). Thus, from an *Enallagma* larva's perspective, waters supporting fish other than centrarchids are probably habitats with low-intensity fish and dragonfly predation.

## MATERIALS AND METHODS

### Adult Male Cercus Morphometrics

To quantify parallel evolution of cercus morphology among species, we compared the cercus morphology of the four Palearctic *Enallagma* species (*E. circulatum*, *E. cyathigerum*, *E. deserti*, and *E. risi*) with the 18 Nearctic *Enallagma* species derived from the two recent radiations (see Turgeon et al. 2005). Source populations and sample sizes are given in Appendix 1 available online at <http://dx.doi.org/10.1554/05-192.1.s1>. *Enallagma* cerci have a fundamental shape that corresponds to a two-tined fork (Fig. 2). We established nine landmark points on the outlines of cerci when viewed laterally and calculated 20 distances among these (Fig. 2). Landmarks 1 and 9 are where the cerci attach to the 10th abdominal segment. The remaining seven landmarks identify points of beginning, maximum, and ending of curves in the two tines of the fork. Interspecific differences in cerci morphology primarily reflect differences in the lengths, widths and displacements of the two tines. All cerci were digitized by M. McPeck using BioScan Optimas software (Media Cybernetics, Silver Springs, MD).

We first performed a UPGMA cluster analysis on the 20 mean distances for the full set of 22 species. This method quantifies the overall degree of similarity among the 22 extant species without regard to their phylogenetic and habitat affinities. In addition, we extracted the first three principal components (PCs) from the correlation matrix among the 20 distances (Morrison 1976). We then plotted the average scores for each species to visualize the relative positions of species in this reduced cercal PC space.

### Larval Morphometrics and Behavior

To quantify ecomorphological variation among species in the larval stage, we compared the morphologies of abdomens, legs, and caudal lamellae for three of the four Palearctic *Enallagma* (*E. circulatum*, *E. cyathigerum*, *E. risi*) with those of closely related Nearctic fish-lake *Enallagma* (*E. vernale*, *E. davisii*, *E. ebrium*, *E. geminatum*, *E. hageni*) and the four Nearctic dragonfly-lake *Enallagma* (*E. annexum*, *E. aspersum*, *E. boreale*, *E. doubledayi*). This dataset was collected by three people over 14 years (Appendix 2, available online at <http://dx.doi.org/10.1554/05-192.1.s2>, gives the source populations, sample sizes, the observer, and the year). Data for three of the species (*E. aspersum*, *E. geminatum*, *E. hageni*) were included in McPeck (1995a). Because the larvae of *E. boreale* and *E. annexum* in this earlier report (McPeck 1995a) could not be unambiguously assigned to species, those data have been excluded here. We present data on *E. boreale* and *E. annexum* from two separate ponds where only one of these species was seen ovipositing the year preceding collection and the year of collection. Ideally, all data would have been digitized by the same person. However, we feel that any observer bias is minimal: for example, the Nearctic dragonfly-lake *E. annexum* and *E. boreale*, which were measured by different people (R. Stoks and J. Nystrom, respectively), show very close morphological similarity in our analyses (see Results).

Our methods are identical to those reported in McPeck

(1995a). Briefly, final-instar larvae with unregenerated legs and caudal lamellae were collected in the field and preserved in 70% ethanol until digitized. Abdomens, back and middle legs, and median and lateral lamellae were digitized using the landmarks presented in McPeck (1995a) using BioScan Optimas software (for a summary of landmarks and distances used, see Appendix 3 available online at <http://dx.doi.org/10.1554/05-192.1.s3>).

We reduced the dimensionality of the large set of morphological variables by performing principal component analyses (PCAs) separately for each of the three structures (abdomen, legs, and lamellae). The resulting PCs describe most of the variation in the original dataset and have the advantage of being uncorrelated. For each structure PC1 and PC2 were extracted from the correlation matrix and rotated with the varimax normalized procedure using Statistica 5.1 software (Statsoft, Tulsa, OK; Morrison 1976). Because less than 5% of the original morphological variables differed significantly from normality, we did not transform them prior to analysis.

We applied univariate nested analyses of variance (NANOVA) with planned contrasts to test for morphological differences among the four species groups (i.e., Nearctic fish-lake *Enallagma* [*E. davisii*, *E. ebrium*, *E. geminatum*, *E. hageni*], Nearctic dragonfly-lake *Enallagma* [*E. annexum*, *E. aspersum*, *E. boreale*, *E. doubledayi*], Nearctic *E. vernale*, and Palearctic *Enallagma* [*E. circulatum*, *E. cyathigerum*, *E. risii*] using the PCs as response variables. The NANOVA model included group and species nested within group as independent variables. To test for group differences, we constructed planned contrasts to test for specific group differences; the species nested within group term was used as the error variance for each contrast (Sokal and Rohlf 1995). The first contrast tested for differences between the Nearctic dragonfly-lake *Enallagma* and the Nearctic fish-lake *Enallagma*; this contrast confirmed the differences between these two groups identified in McPeck (1995a) but including three new species (*E. davisii*, *E. doubledayi*, *E. ebrium*) and different source populations for some species. The critical tests for our current hypotheses contrasted the Nearctic *E. vernale* and the Palearctic *Enallagma* separately with both of these groups to test their morphological similarities and differences with the Nearctic fish-lake and dragonfly-lake groups.

We also evaluated the behaviors of final-instar larvae for Nearctic *E. vernale* and Palearctic *E. cyathigerum* populations from a fish lake and a dragonfly pond, and we compared their baseline activity and predator-induced behavioral plasticity to those of Nearctic dragonfly-lake and fish-lake *Enallagma*. As with the morphometrics, the complete dataset was collected by three people over 17 years (Appendix 4 available online at <http://dx.doi.org/10.1554/05-192.1.s4>). Elsewhere we have discussed that our behavioral results are not biased by an observer/year effect (Stoks et al. 2003). Our procedures here closely followed those of McPeck (1990b), and we include data for two species (*E. aspersum*, *E. geminatum*) used in that study, as well as data reported in Stoks et al. (2003). The behaviors of individual larvae were observed for 30 min in experimental tanks in the presence of: (1) no predators; (2) three *Anax* dragonfly larvae; or (3) one 5- to 6-cm standard length sunfish (*L. macrochirus* used in 1988, *L. gibbosus* used

in other trials). The *Enallagma* larvae were separated from the predators by a transparent Plexiglas wall and given 25 *Daphnia pulex* as prey. We analyzed eight behavioral variables that have been shown to differ strongly between *Enallagma* species from fish lakes and dragonfly lakes: the number and mean duration of walks, the duration of the longest motionless period, the number of orientations and advances toward prey, the number of successful and unsuccessful strikes at prey, and the number of rigid abdominal bends (McPeck 1990b; Stoks et al. 2003).

Although no functional dependency existed between the behaviors, the variable responses are statistically correlated (e.g., decreases in "activity" are reflected in the changes in many behavioral variables simultaneously). Therefore, to reduce the dimensionality of the dataset and to remove correlations among analyzed variables, we first extracted PCs from the correlation matrix of the original variables (Sokal and Rohlf 1995). The resulting three largest PC axes were then rotated using the varimax normalized method to aid their interpretation. Individual scores on these rotated PCs were analyzed to determine species differences and treatment responses. Prior to analysis, the eight original variables were  $\log_e(x + 1)$ -transformed because of clear dependencies between the means and variances of variables across treatments.

We applied univariate analyses of variance (ANOVA) to the PC scores to test a priori hypotheses about the differences among the four groups (i.e., dragonfly-lake *Enallagma*, fish-lake *Enallagma*, *E. vernale*, Palearctic *E. cyathigerum*) and responses to the presence of predators within each group. Because one general model cannot be constructed to test simultaneously hypotheses about group differences and predator treatment differences, we took a two-step approach to this analysis. The first step was to test for group differences comparing only the absence of predators treatment responses in a NANOVA, with group and species nested within group as independent variables in the model. This analysis was analogous to that applied to the morphological data. In the absence of predators, the Palearctic *E. cyathigerum* from fish-lake and dragonfly-lake populations did not differ in any of the behavioral PCs (univariate ANOVAs, all  $P > 0.32$ ); we therefore combined the two populations for this first analysis.

In the second step, we examined responses to the presence of predators for each species group separately. The statistical significance of differences among the three predator treatments were evaluated using planned a priori contrasts in separate ANOVAs for *E. vernale* and each population of Palearctic *E. cyathigerum*. Previous analyses showed that the Nearctic dragonfly-lake *Enallagma* species decrease their behavioral activity (movement and feeding) in the presence of only dragonflies, but the fish-lake *Enallagma* species decrease activity in the presence of both fish and dragonfly predators (McPeck 1990b; Stoks et al. 2003). Based on these differences, we expected *E. vernale* to decrease their movement and feeding in the presence of both predators, and so we tested the contrast set: (1) predator absent versus (dragonflies present + fish present); and (2) dragonflies present versus fish present. This contrast was also used for the fish-lake population of the Palearctic *E. cyathigerum*. Because we expected the Palearctic dragonfly-lake *E. cyathigerum* to respond only to the presence of dragonflies, we tested the con-

trast set: (1) (predator absent + fish present) versus dragonflies present; and (2) predator absent versus fish present.

Because the radiations producing the species under study in this paper occurred so recently, they show little or no sorting of mtDNA lineages into reciprocally monophyletic groups within each radiation. Therefore, we assume a star phylogeny within each radiation. Our linear contrasts analyses test differences among the average species phenotype within and between phylogenetic groups. We feel that the averaging across species within groups that is inherent with linear contrasts is conservative with respect to the phylogenetic affinities of the species included in the study. Moreover, because we cannot resolve the affinities of species within each radiation, performing more sophisticated analyses that correct for phylogenetic affinities (e.g., evolutionary contrasts analyses) add no additional insights beyond the analyses we present here.

## RESULTS

### Adult Male Cercus Morphometrics

The UPGMA clustering of species based on the 20 distances between cercus landmarks shows some degree of phylogenetic pattern, but overall cercus morphology is not a consistent predictor of phylogenetic affinity or larval habitat affinity (cf. Fig. 3A with the phylogenetic relationships reported in Turgeon et al. 2005). By clustering them together, the morphometric analysis supports the qualitative observation that three Paelearctic *Enallagma* (*E. circulatum*, *E. deserti*, and *E. risi*) share the *boreale*-type cerci with the Nearctic *E. boreale* and that the Paelearctic *E. cyathigerum* shares the *cyathigerum*-type cerci with the Nearctic *E. annexum* and *E. vernale* (Figs. 1, 3A). Note that in addition to the latter cryptic species group, *E. clausum* and *E. geminatum* also cluster with the *cyathigerum*-type species. This is because clustering summarizes only distances in a multidimensional space. When viewed in the three-dimensional PC space, *E. cyathigerum*, *E. annexum*, and *E. vernale* clearly form a tight grouping to one another (Fig. 3B PC loadings for these axes are given in Table 1).

### Larval Morphometrics and Behavior

#### Principal components loadings and variable interpretation

The first two PCs extracted from each morphological structure summarized more than 80% the variation in each (Table 2). As in McPeck (1995a), abdomen PC1 had large, positive loadings from the segment lengths and was interpreted as a metric of abdomen length. Abdomen PC2 had large, positive loadings from the abdominal segment widths and was interpreted as a metric of abdomen width. Leg PC1 was interpreted as a size metric, having large, positive loadings from the femur and tibia lengths of both back and middle legs. Leg PC2 had high positive loadings from the femur and tibia widths. Lamellae PC1 was also interpreted as a size metric, having large, positive loadings for the areas and perimeters of both lamellae. Lamellae PC2 quantified shape with large, positive loadings from the circularity of both lamellae; larvae with a higher score for PC2 had less circular lamellae. For the eight behavioral variables, PC1 had high positive loadings

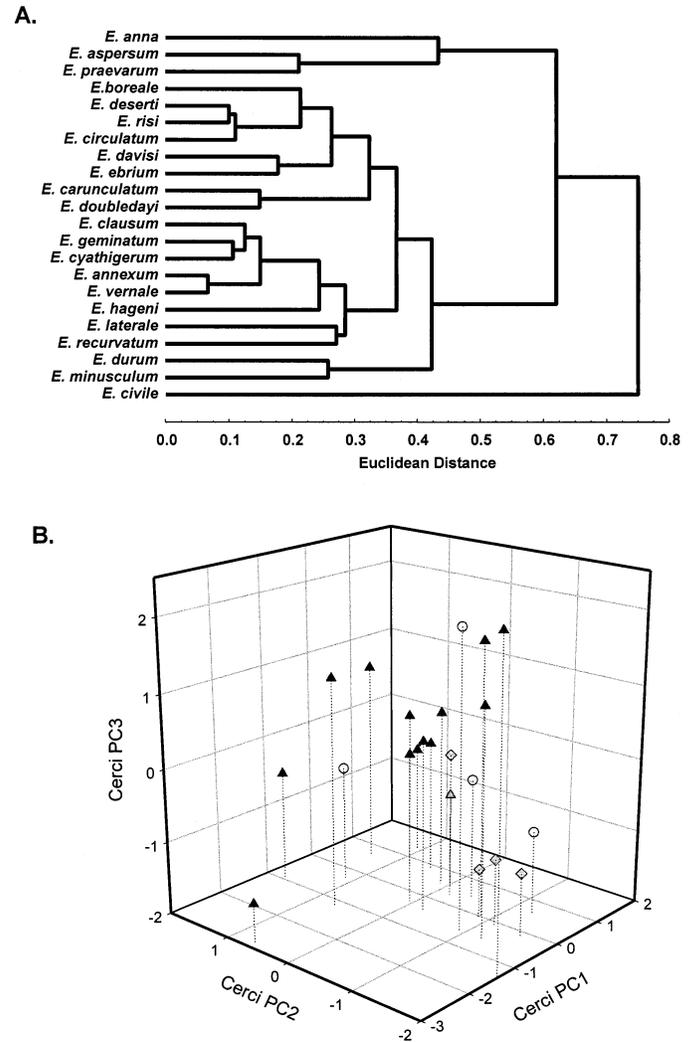


FIG. 3. (A) UPGMA clustering and (B) position in cerci morphological space of the four Paelearctic and 18 closely related Nearctic *Enallagma* species based on the 20 distances among cerci landmarks (see Fig. 2). Branch lengths give the Euclidean distances among clusters. Cerci principal components are defined in Table 1. Fish-lake *Enallagma* are represented by black triangles, except *E. vernale*, which is identified by a gray triangle; dragonfly-lake *Enallagma* by white circles; and Paelearctic *Enallagma* by gray diamonds.

from all variables associated with foraging, behavior PC2 had high positive loadings from the number of abdominal bends and the number of walks, and behavior PC3 was strongly and positively associated with the mean duration of walks (Table 3).

Analyses of these morphological and behavioral datasets identify the same differences between the Nearctic fish-lake and dragonfly-lake *Enallagma* as we have demonstrated in previous studies (McPeck 1990b, 1995a,b; McPeck et al. 1996; Stoks et al. 2003). The major differences between these two species groups are as follows (analyses not shown): (1) dragonfly-lake species have wider abdomens, longer legs, and larger caudal lamellae relative to their overall body size than fish-lake species; (2) in the absence of predators, dragonfly-lake species walk more, perform more rigid abdominal bends, and walk for shorter periods of time than fish-lake species;

TABLE 1. Loadings of the original distances between the landmarks of the cerci on the first three principal components (PC1, PC2, and PC3). For each distance the corresponding landmarks are given in brackets as in Figure 2.

Distance	PC1	PC2	PC3
Distance 1 [1,2]	-0.79	-0.22	0.32
Distance 2 [1,3]	-0.76	-0.45	0.11
Distance 3 [1,4]	-0.75	-0.57	0.02
Distance 4 [1,7]	-0.85	-0.25	0.14
Distance 5 [2,3]	0.05	-0.77	-0.45
Distance 6 [3,4]	0.12	-0.68	-0.03
Distance 7 [2,4]	0.18	-0.85	-0.36
Distance 8 [3,5]	-0.12	-0.41	0.83
Distance 9 [5,7]	-0.40	0.32	0.74
Distance 10 [6,7]	-0.61	0.37	0.15
Distance 11 [7,8]	-0.79	0.38	-0.24
Distance 12 [6,8]	-0.81	0.39	-0.15
Distance 13 [3,9]	-0.89	-0.28	0.26
Distance 14 [6,9]	-0.85	0.31	-0.27
Distance 15 [7,9]	-0.79	0.38	-0.26
Distance 16 [8,9]	-0.45	0.35	-0.17
Distance 17 [3,7]	-0.15	-0.10	0.86
Distance 18 [1,9]	-0.60	-0.41	0.08
Distance 19 [1,5]	-0.71	-0.48	-0.38
Distance 20 [5,9]	-0.86	0.13	-0.41
Percentage of variance explained	41.7	20.0	15.5

and (3) dragonfly-lake species respond only to the presence of dragonfly predators by decreasing their movement and responsiveness to prey, while fish-lake species respond to the presence of both fish and dragonflies by reducing their movement and responsiveness to prey.

#### *Palaearctic Enallagma versus Nearctic fish- and dragonfly-lake Enallagma*

Palaearctic *Enallagma* did not differ in abdomen morphology from dragonfly-lake *Enallagma* (PC1:  $F_{1,8} = 1.23$ ,  $P > 0.30$ ; PC2:  $F_{1,8} = 1.30$ ,  $P > 0.28$ ) but had wider abdomens than fish-lake *Enallagma* (PC1:  $F_{1,8} = 0.57$ ,  $P > 0.47$ ; PC2:  $F_{1,8} = 22.29$ ,  $P < 0.01$ ; Fig. 4A). Palaearctic *Enallagma* also did not differ in leg morphology from dragonfly-lake *Enallagma* (PC1:  $F_{1,8} = 1.68$ ,  $P > 0.23$ ; PC2:  $F_{1,8} = 1.89$ ,  $P > 0.20$ ) but they had longer legs than fish-lake *Enallagma* (PC1:  $F_{1,8} = 14.48$ ,  $P < 0.01$ ; PC2:  $F_{1,8} = 0.50$ ,  $P > 0.49$ ; Fig. 4B; and again after correcting for body size: ANCOVA of PC1:  $F_{1,8} = 16.73$ ,  $P < 0.01$ ). Finally, they did not differ in lamellae morphology from dragonfly-lake *Enallagma* (PC1:  $F_{1,8} = 0.00$ ,  $P > 0.99$ ; PC2:  $F_{1,8} = 0.50$ ,  $P > 0.49$ ) but had larger lamellae than fish-lake *Enallagma* (PC1:  $F_{1,8} = 9.51$ ,  $P < 0.05$ ; PC2:  $F_{1,8} = 0.10$ ,  $P > 0.75$ ; Fig. 4C; and again when corrected for body size: ANCOVA of PC1:  $F_{1,8} = 8.82$ ,  $P < 0.05$ ).

In the absence of predators, Palaearctic *E. cyathigerum* had marginally nonsignificantly shorter walk durations than fish-lake *Enallagma* (PC3,  $F_{1,4} = 6.65$ ,  $P = 0.061$ , Figs. 5E, F; PC1 and PC2; both  $P > 0.23$ ) and did not differ from the dragonfly-lake *Enallagma* in any behavioral variable (all contrasts  $P > 0.11$ ). The dragonfly-lake population of the Palaearctic *E. cyathigerum* only responded to the dragonfly predator and only so by reducing its feeding behavior (PC1:  $F_{1,21} = 76.31$ ,  $P < 0.0001$ , all other contrasts  $P > 0.30$ ). The fish-lake population of the Palaearctic *E. cyathigerum*, however, responded to both predators with reduced feeding (PC1:  $F_{1,21}$

$= 13.34$ ,  $P < 0.01$ ; both other PCs:  $P > 0.21$ , Figs. 5A, B). The responses did not differ between the fish and dragonfly predator treatments, except a somewhat lower number of abdominal bends and walks in the presence of the dragonfly predator compared to the fish (PC2:  $F_{1,21} = 5.91$ ,  $P < 0.05$ , all other  $P > 0.30$ ).

These comparisons show that Palaearctic *E. cyathigerum* are indistinguishable in larval morphology from Nearctic dragonfly-lake *Enallagma* and differed from Nearctic fish-lake *Enallagma* in ways similar to Nearctic dragonfly-lake *Enallagma*. Larvae from the dragonfly-lake population were also behaviorally similar to dragonfly-lake *Enallagma*, while larvae from the fish-lake population were behaviorally similar to fish-lake *Enallagma*.

#### *Comparisons of Nearctic Enallagma vernale to Nearctic fish- and dragonfly-lake species*

*Enallagma vernale*, the *cyathigerum*-type cerci species that coexists as larvae with fish, did not differ in abdomen morphology from dragonfly-lake *Enallagma* (PC1:  $F_{1,8} = 0.33$ ,  $P > 0.58$ ; PC2:  $F_{1,8} = 0.16$ ,  $P > 0.70$ ) but had a wider abdomen than fish-lake *Enallagma* (PC1:  $F_{1,8} = 0.11$ ,  $P > 0.74$ ; PC2:  $F_{1,8} = 15.47$ ,  $P < 0.01$ ; Fig. 4A). It also did not differ in leg morphology from dragonfly-lake *Enallagma* (PC1:  $F_{1,8} = 1.64$ ,  $P > 0.23$ ; PC2:  $F_{1,8} = 0.27$ ,  $P > 0.61$ ) but had longer legs than fish-lake *Enallagma* (PC1:  $F_{1,8} = 15.49$ ,  $P < 0.01$ ; PC2:  $F_{1,8} = 0.40$ ,  $P > 0.54$ ; Fig. 4B), also after correcting for body size (PC1:  $F_{1,8} = 18.95$ ,  $P < 0.01$ ). Finally, it did not differ in lamellae size from dragonfly-lake *Enallagma* (PC1:  $F_{1,8} = 2.08$ ,  $P > 0.18$ ) but had larger lamellae than fish-lake *Enallagma* (PC1:  $F_{1,8} = 6.22$ ,  $P < 0.05$ ; Fig. 4C), also after correction for body size ( $F_{1,8} = 6.37$ ,  $P < 0.05$ ). It had less circular lamellae than dragonfly-lake *Enallagma* (PC2:  $F_{1,8} = 5.70$ ,  $P < 0.05$ ) and fish-lake *Enallagma* (PC2:  $F_{1,8} = 6.37$ ,  $P < 0.05$ ).

In the absence of predators, *E. vernale* did not differ from

TABLE 2. Loadings of the original morphometric variables of the abdomen, back and middle legs, and median and lateral caudal lamellae on the first two principal components (PC1 and PC2). Abbreviations are given in Appendix 3 (available online).

Abdomen variable	Abdomen		Leg variable	Leg		Lamellae variable	Lamellae	
	PC1	PC2		PC1	PC2		PC1	PC2
ABD1	0.49	0.76	BL1	0.67	0.53	MC1	0.80	0.15
ABD2	0.62	0.38	BL2	0.90	0.40	MC2	0.79	0.61
ABD3	0.60	0.38	BL3	0.90	0.39	MC3	0.79	0.60
ABD4	0.51	0.80	BL4	0.69	0.53	MC4	0.74	0.47
ABD5	0.81	0.29	BL5	0.58	0.57	MC5	0.74	0.48
ABD6	0.78	0.35	BL6	0.91	0.39	MC6	0.69	0.52
ABD7	0.50	0.76	BL7	0.91	0.38	MCA	0.96	0.23
ABD8	0.87	0.25	BL8	0.43	0.70	MCW	0.93	-0.26
ABD9	0.87	0.35	BL9	0.18	0.81	MCC	0.01	0.96
ABD10	0.39	0.89	ML1	0.71	0.54	MCL	0.81	0.57
ABD11	0.87	0.33	ML2	0.90	0.41	MCP	0.97	0.22
ABD12	0.87	0.35	ML3	0.91	0.37	LC1	0.88	0.18
ABD13	0.32	0.92	ML4	0.70	0.46	LC2	0.82	0.56
ABD14	0.86	0.38	ML5	0.59	0.55	LC3	0.81	0.57
ABD15	0.86	0.39	ML6	0.91	0.40	LC4	0.74	0.67
ABD16	0.29	0.92	ML7	0.91	0.38	LC5	0.74	0.68
ABD17	0.81	0.44	ML8	0.55	0.58	LC6	0.69	0.52
ABD18	0.81	0.45	ML9	0.35	0.73	LCA	0.96	0.23
ABD19	0.29	0.90				LCW	0.93	-0.26
						LCC	0.01	0.96
						LCL	0.81	0.57
						LCP	0.97	0.52
Percentage of variance explained	0.47	0.35		0.55	0.27		0.61	0.29

dragonfly-lake or fish-lake *Enallagma* on any of the three behavioral PCs (all  $P > 0.10$ , Fig. 5). When placed in the presence of predators, however, *E. vernale* larvae did respond to both predators with reduced feeding (PC1:  $F_{1,21} = 13.34$ ,  $P < 0.01$ ; both other PCs:  $P > 0.20$ , Fig. 5).

These comparisons show that *E. vernale* is, except for lamellae shape, indistinguishable in morphology from the Nearctic dragonfly-lake species. *Enallagma vernale* is, however, behaviorally intermediate to the two groups in the absence of predators, and it responds to the presence of fish like other fish-lake species.

#### DISCUSSION

Our results suggest striking parallelism to two separate cerci types in sets of Nearctic and Palearctic species. If either *E. hageni* or *E. ebrium* is the progenitor from which the Nearctic *E. boreale*, *E. annexum*, and *E. vernale* are derived (Turgeon and McPeck 2002; Turgeon et al. 2005), the cerci morphologies of the Nearctic and Palearctic counterparts within each set of cryptic species must be independently derived. Even assuming that the original Palearctic colonist had

one of these two cerci types, this means that the same cerci morphologies must have independently evolved within the Holarctic *Enallagma* at least three times. Such parallel trait evolution involved in mate recognition has been documented recently in other insect groups (e.g., lacewings, Henry et al. 1999; sepsid flies, Eberhard 2001; and fruitflies, Kopp and True 2002) and is thought to be driven by sexual selection via female choice (Eberhard 1985). Parallel changes of both cerci types in the Nearctic and Palearctic clade is most likely the result of the relative simplicity of the underlying genetic architecture and developmental machinery causing some cerci types to be more accessible through fewer genetic changes than others (Henry et al. 1999; Eberhard 2001; Orr 2001).

In North America, the recent radiations of *Enallagma* have been well characterized both phylogenetically and ecologically (e.g., McPeck and Brown 2000; Turgeon et al. 2005). Several possible scenarios can be imagined that could have resulted in the present-day Nearctic *E. annexum*, *E. boreale*, and *E. vernale* from one of these radiations, but only one is consistent with the phenotypes and habitat distributions of these three species. In this scenario we hypothesize that the

TABLE 3. Loadings of the original behavioral variables on the first three principal components.

Behavioral variable	PC1	PC2	PC3
Number of walks	0.28	0.75	0.43
Mean duration of walks (sec)	0.03	0.02	0.97
Duration of longest motionless period (min)	-0.69	-0.56	-0.20
Number of orientations of the head and body toward prey	0.93	0.10	0.08
Number of advances toward prey	0.84	0.01	0.03
Number of unsuccessful strikes at prey	0.75	0.22	0.09
Number of prey captured	0.86	0.13	-0.02
Number of abdominal bend displays	0.03	0.91	-0.17
Percentage of variance explained	0.43	0.22	0.15

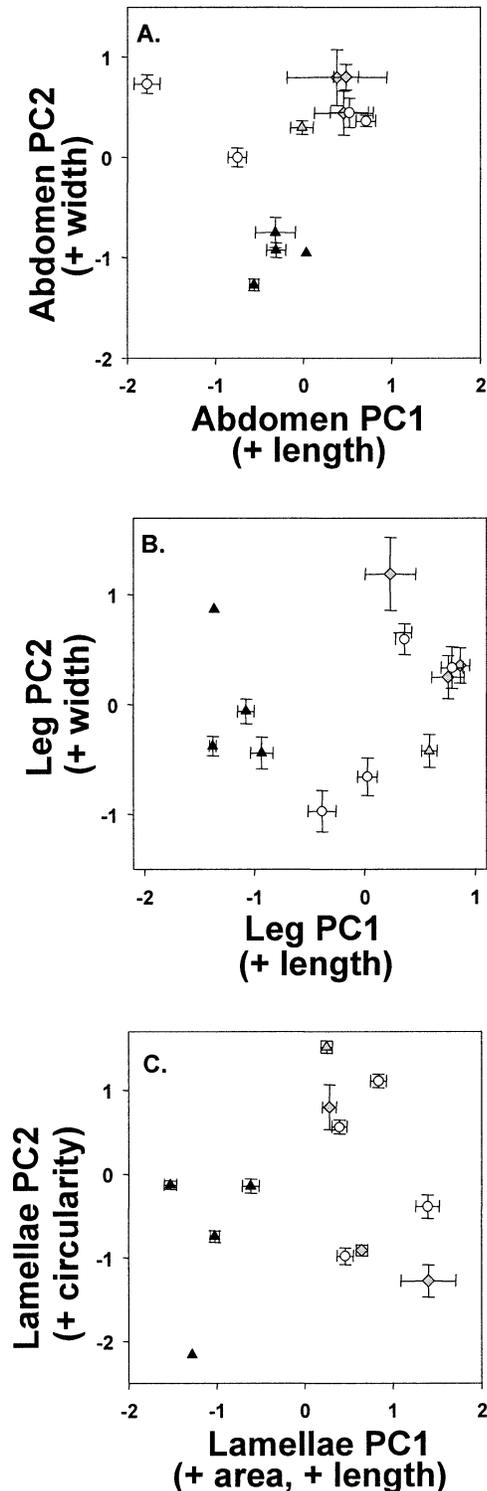


FIG. 4. Mean ( $\pm 1$  SE) scores for the morphological principal components associated with the abdomen (A), legs (B), and lamellae (C) for three Palearctic and eight closely related Nearctic *Enallagma* species. Symbol codings are as in Figure 3.

first event to have occurred was the adaptation of a fish-lake lineage to live under the predation regime found in the dragonfly-lake habitat. This shift would have produced the morphological, behavioral, and biochemical changes found in the two dragonfly-lake species today. Following this adaptive habitat shift, the lineage would have subsequently speciated to produce the present *E. annexum* and *E. boreale* (Turgeon et al. 2005). Given their nearly identical behavioral and morphological phenotypes, their extensive sympatry in dragonfly ponds and lakes on a local scale, and their nearly coincident ranges that cover much of North America, the speciation event that produced *E. annexum* and *E. boreale* most likely occurred primarily by differentiation of the mate recognition system (i.e., generating differences in cerci morphology).

Subsequent to this split, we hypothesize that *E. vernale* was then derived from *E. annexum* in the eastern part of its range; *E. vernale* is now found in fish waters from New England to Michigan (Donnelly 1989). In our experience, larvae of *E. vernale* are found in fish lakes with other fish-lake *Enallagma*. *Enallagma vernale* is nearly indistinguishable from *E. annexum* and *E. boreale* in larval morphological features (wide abdomens, large lamellae, and long legs; Fig. 4), but *E. vernale*'s response to reduce feeding in the presence of both fish and dragonflies is identical to the fish-lake species with which it currently coexists (Fig. 5). Morphology plays no role in avoiding fish predators, and so we cannot propose a credible hypothesis for why *E. vernale* would share the morphological features of the dragonfly-lake species while remaining in the fish-lake environment. However, reducing activity in the presence of fish is critical to avoiding fish predation (Pierce et al. 1985; Stoks and Johansson 2000; Stoks et al. 2005). Based on these morphological and behavioral relationships, we hypothesize that *E. vernale* is the result of a secondary reinvasion to what was the original ancestral habitat for the genus. The change in selective environment by reinvading fish lakes would have only focused selection pressures to decrease overall activity and reduce activity in the presence of fish, not alter its morphology (McPeck 1990b; Stoks et al. 2003). Thus, we believe the simplest explanation for *E. vernale*'s chimeric phenotype (i.e., morphology of a dragonfly-lake species and behavior largely of a fish-lake species) is by inheritance of morphology from a dragonfly-lake ancestor (namely *E. annexum*) and selection to alter its behavior in the presence of fish predators.

In contrast, Palearctic *Enallagma* larvae live in both fish lakes and dragonfly lakes, and the ecological reasons for their apparently greater ecological breadth remains unclear. However, the morphological and behavioral phenotypes identified in this study provide interesting and testable hypotheses about intercontinental community ecology and local adaptation. The morphological similarity of Palearctic *E. cyathigerum* and the Nearctic dragonfly-lake species (Fig. 4) suggests that dragonfly predation has also played a key role in the history of this lineage. Again, such morphologies are favored by intense dragonfly predation (McPeck 1997) but not by any selection pressures that are associated with fish lakes. Given that either *E. hageni* or *E. ebrium* was the progenitor of the radiation that gave rise to the Nearctic *E. annexum* and *E. boreale* (Turgeon and McPeck 2002; Turgeon et al. 2005), none of the extant Nearctic dragonfly-lake spe-

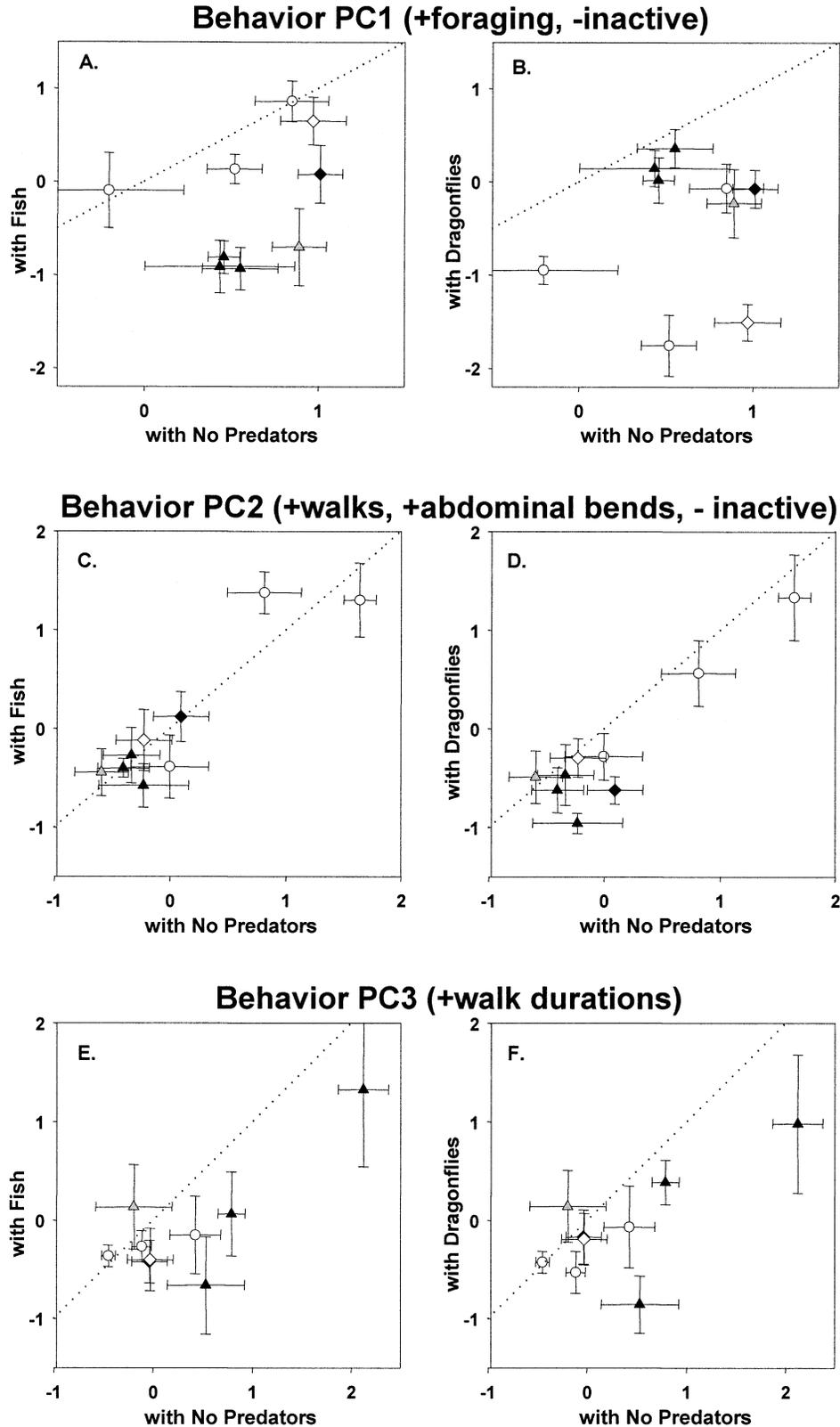


FIG. 5. Mean ( $\pm 1$  SE) scores for the three behavioral principal components (PCs) for one Palearctic and six closely related Nearctic *Enallagma* species under the three predator treatments (no predator, fish, and dragonfly predator). The left panels plot the behavioral PCs in the presence of fish against the presence of no predators, and the right panels plot the behavioral PCs in the presence of dragonflies against the presence of no predators. The dotted line identifies the 1:1 relationship between two predator treatments. Brief descriptions of each PC are given by the axis labels (see Table 3). Symbol codings are as in Figure 3, except that the fish-lake and dragonfly-lake populations of Palearctic *E. cyathigerum* are identified with filled and open diamonds, respectively.

cies could have been the original colonist of the Palearctic. Either Palearctic *Enallagma* larval morphology was inherited directly from the original colonist (if the original Palearctic colonist was a Nearctic dragonfly-lake species that is now extinct), or the original Palearctic colonist was a fish-lake species that underwent a shift into the dragonfly-lake habitat before the Palearctic clade began to diversify. Under both of these scenarios the striking similarity of larval dragonfly-lake morphologies among the Nearctic and Palearctic species despite their independent origin implicates their parallel evolution driven by dragonfly predation.

The affinity of Palearctic *Enallagma* species with both fish and dragonfly lakes is similar to the lack of segregation of species among these lake types in western North America. In eastern North America, where *Lepomis* species (Centrarchidae) are the dominant littoral fish predators, *Enallagma* species show a checkerboard distribution among fish and dragonfly lakes (Johnson and Crowley 1980; McPeck 1990a, 1998). Moreover, when *Lepomis* species are introduced into a dragonfly lake, the native dragonfly-lake *Enallagma* are extirpated quickly (M. A. McPeck, pers. obs. of numerous fish introductions across eastern North America). In western North America outside of the native range of *Lepomis*, species that segregate between fish lakes and dragonfly lakes in eastern North America can be found together in most lakes with fish (Anholt 1990; Chivers et al. 1996; M. A. McPeck, unpubl. data; D. R. Paulson, pers. comm.). We hypothesize that the fish found in western lakes are less effective littoral predators than centrarchids and as a result do not impose enough mortality to exclude any *Enallagma* species from coexisting with them. *Enallagma annexum* and *E. boreale* larvae from these western populations are capable of learning about threatening fish predators (Chivers et al. 1996; Wisenden et al. 1997). If larvae from eastern populations are similarly capable of learning, it is not adequate to prevent their extirpation by *Lepomis* species. Moreover, populations of large dragonfly species (e.g., *Anax* and *Aeshna*) that dominate fishless waters may also be able to maintain significant populations in the presence of fish in these western lakes. Consequently, the predation regimes in western fish lakes may be less intense but mechanistically more diverse than in eastern fish lakes, where *Lepomis* dominate. Thus, the selection pressures on individual species and the dynamics of the entire littoral food web may be quite different in eastern versus western North American fish lakes.

The same can be said of the Palearctic, since centrarchid fishes are endemic to North America, and the western North American and Eurasian fish faunas are similar. In eastern North America, *Lestes* and *Coenagrion* species, as well as *Enallagma* species, segregate between fish and dragonfly lakes (Johnson and Crowley 1980; Stoks and McPeck 2003), but Palearctic members of all these genera show no strong segregation between fish and dragonfly waters (Corbet 1999; Samraoui et al. 2002; Johansson and Brodin 2003; R. Stoks, pers. obs.). Predation regimes in fishless ponds and lakes should be dominated by dragonfly predation all across the Palearctic and Nearctic. Dragonfly predation may thus be the dominant selection pressure when averaged across all populations of *Enallagma* species in both the Palearctic and western North America because of less effective fish predators.

This may explain why ecomorphological characteristics of the Palearctic *Enallagma* species are similar to Nearctic species in dragonfly lakes (Fig. 4). However, populations from fish and dragonfly lakes did differ in their behavioral responses to fish predators (Fig. 5). *Enallagma annexum* larvae from New England populations that have been reared in the laboratory from eggs are innately naive that fish are predators (F. Strobbe, R. Stoks, and M. A. McPeck, unpubl. data). These population differences may therefore not simply be the result of learning, as in the western Nearctic *E. boreale* and *E. annexum* populations. Although potentially devastating to the local aquatic faunas, the recent introductions of *Lepomis* species to Japan and parts of Europe ironically may provide an unfortunate test of these conjectures (e.g., Welcomme 1988; Economidis et al. 2000; Garcia-Berthou and Moreno-Amich 2000).

We have presented evidence for parallel evolution in larval ecomorphology in the genetically and geographically separated Nearctic dragonfly-lake *Enallagma* and in the Palearctic *Enallagma*. Earlier studies showed that predation by large dragonfly larvae are the major common selective force that drove these adaptive responses in three independent habitat shifts in the Nearctic *Enallagma* (McPeck 1995a,b, 1997; McPeck et al. 1996). This intriguing parallel evolution of ecomorphs in genetically distinct taxa under similar selection pressures on different continents has recently also been shown in Anura (Bossuyt and Milinkovitch 2000), Cetacea (Cassens et al. 2000), and Chiroptera (Alvarez et al. 1999; Ruedi and Mayer 2001). However, in these other cases morphological differences among taxa were still so obvious that they were never considered to be conspecifics. The independent evolutionary origins of larval ecomorphs that were presumably adapting to similar ecologies and the parallel changes of adult male appendages involved in reproductive isolation led to nearly indistinguishable cryptic species of independent origin in the Nearctic and Palearctic. Interestingly, the Nearctic *E. annexum* and the Palearctic *E. cyathigerum* have long been known as a textbook example of a single Holarctic species (Cox and Moore 1993).

Cryptic species are typically sibling species that diverged in reproductive traits but not in ecologically important traits, and many examples exist in which lineages may have maintained such phenotypic stasis in apparent sympatry for very long periods of time (e.g., Henry et al. 1999; Witt and Hebert 2000; Colborn et al. 2001; Gomez et al. 2002; Hebert et al. 2004). For this kind of cryptic species, phenotypic stasis in nonmating life stages is not necessary. For example, up to 10 species make up a cryptic group of skipper butterflies with extremely similar adults, probably due to stabilizing selection linked to mimicry, but they have larval stages that can be discriminated easily based on color pattern and food plant choice (Hebert et al. 2004). Less well documented are the situations where strong parallel evolution produced cryptic species. Indeed, for almost all cases of parallel evolution, species can still be easily identified. The present finding of two cryptic species sets due to parallel evolution in animals with a complex life cycle where larvae and adults do not share the same selective environment may therefore be rare. Unfortunately, for many cryptic taxa with a complex life cycle that evolved similar traits involved in mate recognition

we have no data on whether larval traits similarly followed parallel tracks (e.g., Henry et al. 1999). Parallel convergence in larval and adult traits has been documented in the morphologies of ranid frogs of Madagascar and Asia (Bossuyt and Milinkovitch 2000). However, in the ranid system both adults and larvae adapted to the same habitats, whereas in the *Enallagma* the differences in selective environment that the larvae experience between fish lakes and dragonfly lakes disappear in the terrestrial adult stage. Moreover, in the ranid frogs convergence was not so striking to result in cryptic species. Unraveling the molecular basis of larval adaptation to various predation regimes and the diversification of specific mate recognition will shed light on whether the observed parallel changes in the larval and adult stage are merely a factor of chance combined with a limited number of evolutionary outcomes, or whether the same genes drive the evolution of both larval and adult stages. The fact that two other habitat shifts into the dragonfly-lake environment produced dragonfly-lake ecomorphs without either the *boreale*- or *cyathigerum*-type cerci (i.e., *E. doubledayi* and *E. aspersum*; Brown et al. 2000; Turgeon et al. 2005) at least implies that the parallel evolution of larval morphology and adult cerci types are not necessarily linked.

Molecular studies are revolutionizing our understanding of biodiversity by identifying many cryptic species. Many taxa that were once thought to be widely distributed single species have been shown to be complexes of morphologically nearly identical species (e.g., Henry et al. 1999; Lee 2000; Witt and Hebert 2000; Mayer and Von Helversen 2001; Gomez et al. 2002; Hebert et al. 2004). We are only beginning to contemplate the genesis of these species and their ecological roles in the environment (McPeck and Gomulkiewicz 2005; Leibold and McPeck 2005). Integrated studies of molecular phylogenetics, natural selection, population regulation, and reproductive differentiation will be needed to understand the ever-greater complexity that is being revealed about our world's biodiversity.

#### ACKNOWLEDGMENTS

We thank everyone who provided us with specimens or information about larval morphology: T. Andries, T. Aoki, R. Bernard, P. Buczynski, G. Carchini, A.C. Cordero, M. De Block, H. J. Dumont, K. Hayashi, K. Inoue, R. Jödicke, F. Johansson, O. Kosterin, N. Matushkina, H. Naraoka, D. Paulson, K. Reinhardt, B. Samraoui, and R. Seidenbusch. Without their generous help, this study would not have been possible. Comments by K. Peterson and two anonymous reviewers helped to greatly clarify the presentation. Financial support was provided by a Francqui Foundation Fellowship in association with the Belgian American Educational Foundation and a postdoctoral fellowship, travel grants, and research grant G.0.260.04 by the Fund for Scientific Research Flanders, and KULeuven Research Fund OT/04/23 to RS, National Science Foundation grant IBN-0130021 to MAM, and a Research Experiences for Undergraduates supplement from the National Science Foundation to support JLN.

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