Hydrobiologia **480:** 111–128, 2002.
C.E. Lee, S. Strom & J. Yen (eds), Progress in Zooplankton Biology: Ecology, Systematics, and Behavior.

Morphological stasis in the *Eurytemora affinis* species complex (Copepoda: Temoridae)

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Key words: cryptic speciation, species concepts, hybrid breakdown, copepod systematics, phylogenetics, morphometrics, allozyme, genetic architecture

Abstract

Morphological stasis has long been regarded as one of the most challenging problems in evolutionary biology. This study focused on the copepod species complex, *Eurytemora affinis,* as a model system to determine pattern and degree of morphological stasis. This study revealed discordant rates of morphological differentiation, molecular evolution, and reproductive isolation, where speciation was accompanied by lack of morphological differentiation in secondary sex characters. Comparisons were made among phylogenies based on morphometrics, nuclear (allozyme) loci, and mitochondrial DNA (mtDNA) sequences from cytochrome oxidase I, for a total of 43 populations within the complex. These systematic relationships were also compared to patterns of reproductive isolation. In addition, genetic subdivision of nuclear molecular (allozyme) markers (G_{ST}) and quantitative (morphological) characters (Q_{ST}) were determined to infer evolutionary forces driving morphological differentiation. The morphometric phylogeny revealed that all clades, excluding the European clade, were morphologically undifferentiated and formed a polytomy (multifurcation). Morphometric distances were not correlated with mtDNA distances, or with patterns of reproductive isolation. In contrast, nuclear and mtDNA phylogenies were mostly congruent. Reproductive isolation proved to be the most sensitive indicator of speciation, given that two genetically and morphologically proximate populations showed evidence of hybrid breakdown. Quantitative genetic (morphological) subdivision (Q_{ST} = 0.162) was lower than nuclear genetic subdivision (G_{ST} = 0.617) for four laboratory-reared North American populations, indicating retarded evolution of morphological characters. This result contrasts with most other species, where Q_{ST} typically exceeds G_{ST} as a result of directional selection. Thus, in all but the European populations, evolution of the secondary sex characters was marked by morphological stasis, even between reproductively-isolated populations.

Introduction

Morphological stasis, the maintenance of a standard morphology over vast periods of time, has been regarded as one of the most challenging problems in evolutionary biology (Darwin, 1872; Gould & Eldredge, 1977; Charlesworth et al., 1982; Wake et al., 1983). Hypotheses on causes of morphological stasis historically have been developed within the field of paleontology, where the concept of morphospecies has prevailed (Simpson, 1944; Lieberman & Dudgeon, 1996; Sheldon, 1996). Within such a context, morphological stasis has been equated commonly with evolutionary stasis, and morphological change with speciation. For example, tempo and mode of morphological evolution have been used to test models of speciation, such as punctuated equilibrium *versus* phyletic gradualism (Gould, 1982). Such models are difficult to evaluate while distinctions between morphological and evolutionary stasis have remained unclear (Charlesworth et al., 1982). With the advent of molecular genetic techniques, many recent studies have begun testing morphological stasis against rates of molecular evolution (Wake et al., 1983; Jackson & Cheetham,

1994; Colbourne & Hebert, 1996; Taylor et al., 1996; King & Hanner, 1998; Müller et al., 2000). Additionally, some studies have begun examining morphological stasis against patterns of reproductive isolation, in addition to rates of molecular evolution, to determine relationships between morphological change and speciation (Jackson & Cheetham, 1990; Palmer et al., 1990), but such studies are still relatively rare. One such study on two cheilostome bryozoan genera found congruence among multiple measures of evolution (morphological, molecular, reproductive isolation), and found the concept of morphospecies applicable (Jackson & Cheetham, 1990).

However, rates of morphological evolution and speciation are likely to be incongruent for many species. Molecular genetic analyses have revealed the prevalence of cryptic speciation in marine invertebrates, resulting in excessive lumping in systematic classifications (Knowlton, 2000). In particular, genetic and morphological studies within the crustacean class Copepoda have uncovered large genetic divergences within morphologically conserved species (Frost, 1974; Frost, 1989; Sevigny et al., 1989; McKinnon et al., 1992; Bucklin et al., 1995; Burton, 1998). The few studies which performed interpopulation crosses among morphologically indistinct populations found incidences of hybrid breakdown and outbreeding depression (Burton, 1990; Ganz & Burton, 1995; Edmands, 1999; Lee, 2000).

This study expands on previous work by Lee (2000), and focuses on the relationship between morphological stasis and rates of speciation in the copepod *Eurytemora affinis* (Poppe, 1880). This copepod has been regarded as a cosmopolitan euryhaline species, with a broad geographic range within the Northern Hemisphere, spanning coastal regions of North America, Asia, and Europe (Fig. 1). However, this 'species' is actually a species complex composed of genetically divergent and physiologically distinct populations (or subspecies), with habitat transitions, such as freshwater invasions, entailing rapid evolutionary events (Lee, 1999; Lee and Bell, 1999; Lee, 2002; Lee & Petersen, 2002). Along with large genetic divergences, reproductive isolation was found between morphologically undifferentiated populations (Lee, 2000).

In this study, morphometric measurements from all clades within this species complex were compared to molecular genetic data and patterns of reproductive isolation. The objective was to address the following questions: **(1)** Are rates of morphological evolution, molecular evolution, and reproductive isolation congruent? **(2)** Which species concept would apply for this species complex? **(3)** What is the pattern of morphological evolution in this species complex, and what evolutionary forces drive that pattern?

To address the first and second questions, phylogenetic patterns based on morphometrics, nuclear loci, and mitochondrial DNA sequences were compared to one another and to patterns of reproductive isolation. In addition, Mantel's test (Mantel, 1967) was used to test correlations between morphometric, nuclear genetic, and mitochondrial distances. The morphological phylogeny was constructed using secondary sex characters, typically used for systematics of this group. The mitochondrial phylogeny was adapted from Lee (2000), while the nuclear phylogeny was based on five allozyme loci. Patterns of reproductive isolation were taken from Lee (2000). To explore the third question, genetic subdivision of nuclear molecular markers (allozymes; G_{ST}) and quantitative (morphological) characters (Q_{ST}) were determined for both wild and laboratory-reared populations in order to infer evolutionary forces driving morphological diversification (Lynch et al., 1999).

Methods and materials

Population sampling

Eurytemora affinis (Poppe, 1880) was collected between 1994 and 1999 from 43 sites spanning much of the global range of the species complex (Fig. 1), including diverse habitats such as hypersaline marshes, brackish estuaries, and freshwater lakes. The congener *E. americana*, used as an outgroup species, was collected from the Duwamish River, WA, USA. Freshwater populations are recent invasions from saline sources within the same drainage system (Lee, 1999). Numbers in Figure 1 refer to populations that were included in the previous study on speciation (Lee, 2000), while letters refer to populations newly added for this study.

Morphometric characters

Secondary sex characters typically used for copepod systematics were measured for this study. The morphometric characters included the genital segment of the female, fifth legs of the male, and antennules of both sexes, some of which were scaled to body length (prosome length). More specifically, characters from the female included genital segment width/prosome

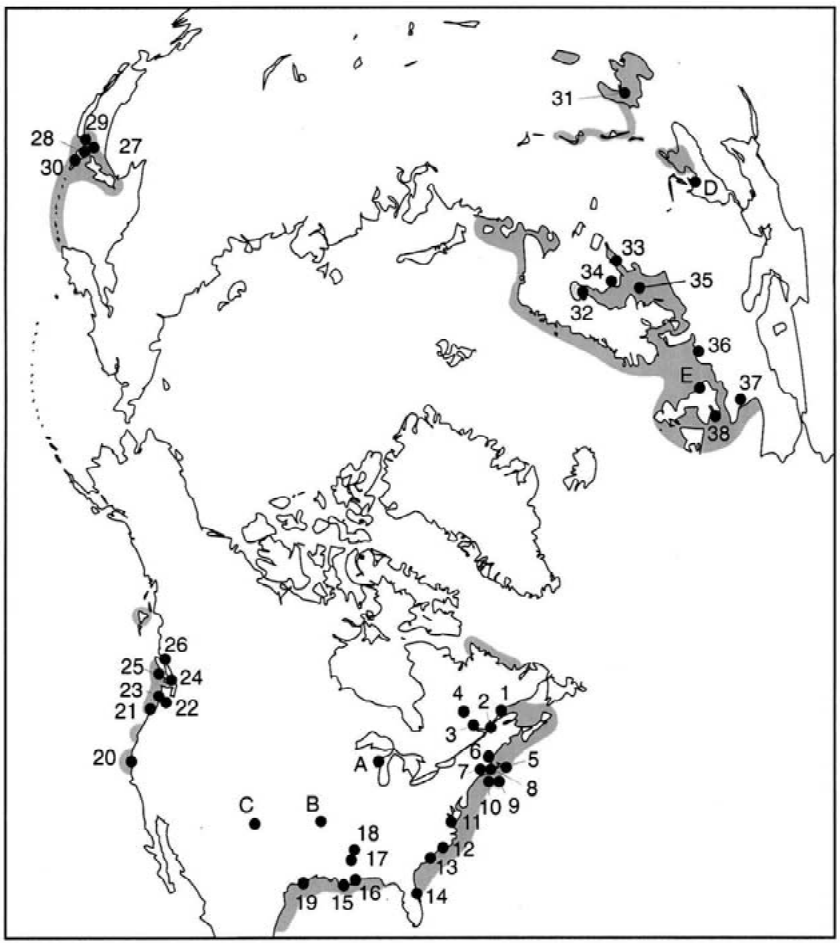

Figure 1. Populations of the *E. affinis* species complex sampled for this study (represented by black dots on map). Gray shading shows the known distribution of *E. affinis*. Distribution of *E. affinis* in northern Russia might be more widespread. Numbers represent populations included in the previous study by Lee (2000), while letters represent additional populations included for this study. Numbers and letters correspond to populations shown in the phylogenies in Figures 3, 4, and 5. Adapted from Lee (2000).

length (GSW/PL), genital segment width/genital segment length (GSW/GSL), and ratios of the 22^{nd} and 24^{th} segments of the first antennule (A1 22:24). Characters from the male included right exopod 1 length/prosome length (rtP5 exo1/PL), right exopod 2 length/prosome length (rtP5 exo2/PL), left basipod 2 width/prosome length (lftP5 Bp2W/PL), right exopod 1 length/left basipod 2 width (rtP5 exo1/lft P5 Bp2W), and ratios of the 22^{nd} and 24^{th} segments of the first left antennule (A1 22:24). These characters are involved in mating or reproduction. The female genital segment contains the genital pore which receives spermatophores from the male and from which eggs are deposited. The male fifth legs are used to attach spermatophores to the female genital segment. The male right antennule is used to capture and grasp onto the female, but the left anntenule was used in this study because its proportions were more consistent and easier to measure. While the female antennule is not used for mating, its pattern of evolution might be correlated with that of the male.

Adult individuals ranged in size among populations from 1 mm to about 2 mm. Prosome length (PL) was measured (60 or 100x) in lateral view from anterior-most point on the cephalosome to posterior margin of the articulating membrane between metasome and urosome. Genital segment length (GSL) of females, taken on unflexed specimens only, was measured (200x) in lateral view along the dorsal side from the anterior margin at the articulating membrane to the posterior edge delimited by a distinct thinning of the cuticle. Genital segment width (GSW) of females

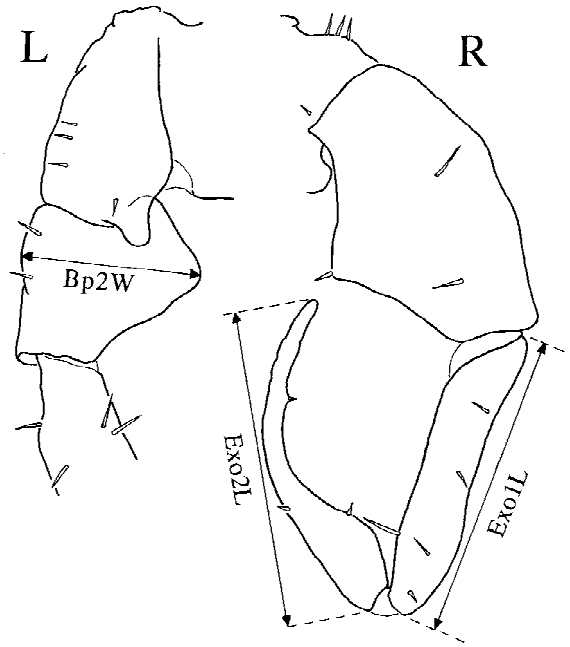

Figure 2. Morphometric measurements for the male fifth leg (P5) of *E. affinis*. Posterior view illustrating the limits of length measurements of individual segments. L: left basipod 2 (Bp2); R: right exopod 1 (Exo1). Modified from Figure 10, Tafel XI of Schmeil (1896).

was measured (200x) dorsally as the maximum width across the dorso-lateral protuberances. In both sexes, lengths of first antennule (A1) segments 22 and 24 (A1 22:24) were measured (400x) along the posterior margin of the dissected appendage. In adult males, fifth leg (P5) measurements (400x) were taken from the posterior side after dissecting the P5 and separating the two legs. Lengths of the right exopod 1 (rtP5 exo1) and right exopod 2 (rtP5 exo2) were measured along the outer margins (Fig. 2). Left basipod 2 width (lft P5 Bp2W) was measured at the point of greatest width (Fig. 2) after removing exopod 2 and part of exopod 1 so that basipod 2 lay flat. Three to five individuals per population were measured for the 23 wild populations, and seven individuals per population were measured for the four laboratory-reared populations.

Morphological phylogeny

The morphological phylogeny was based on the eight characters described above for 23 wild-caught populations (Appendix II). Canonical variates (Rempe and Weber, 1972; Campbell and Atchley, 1981) of morphometric traits were obtained by adapting code (canon9.m) of L. F. Marcus (www.qc.edu/Biology/fac_stf/marcus/multisyl/Tercero.html). A phylogeny based on canonical variates was generated using maximum likelihood with the program CONTML and then bootstrapped using SEQBOOT, within the software package PHYLIP version 3.573c (Felsenstein, 1991). Canonical variates, rather than actual measurements, were used for the phylogenetic reconstruction to remove correlation among characters, because CONTML assumes independence of characters. There has been considerable debate in the literature regarding the validity of using continuous morphometric characters for reconstructing phylogenies (Felsenstein, 1988). In this study, the purpose of the morphometric phylogeny was not to obtain a 'true' phylogeny indicative of evolutionary history, but to make gross comparisons with the molecular phylogenies and display the pattern of morphological differentiation.

Mitochondrial phylogeny

The phylogeny in Figure 3, adapted from Lee (2000), was constructed using the mitochondrial cytochrome oxidase I gene (COI, 652 base pairs). The following extraction protocol was used in both Lee (1999) and Lee (2000), but is elaborated here because of frequent requests for extraction techniques from small tissue samples. A cell-lysis extraction method (Hoelzel and Green, 1992) was modified to extract DNA from individual copepods. Ethanol-preserved copepods were rinsed with TE (pH 8.5) and then placed in 500 μl tubes with 20-40 μl of lysis buffer (10 mm Tris pH 8.3, 50 mm KCl, 0.5% Tween 20, and 200 μg/ml Proteinase K). The tubes were then incubated at 65°C for 1 hour or at 37°C overnight. After incubation, samples were heated at 95°C for 15 minutes to denature the Proteinase K. Samples were then spun in a centrifuge to pellet out debris. The supernatant was stored at −20°C. PCR reactions used 1 μl of the extract or less. PCR conditions are outlined in Lee (2000).

Nuclear phylogeny

Phylogenetic relationships were not based on mitochondrial data alone, but included nuclear loci (allozymes) so that reticulate patterns might be detected (such as from hybridization and introgression among clades). Individual copepods from eleven North American populations were scored for variability in enzyme mobility at five polymorphic loci. For each population, sample size consisted of 20 to 70 individuals,

which were live or frozen at −80°C. Cellulose-acetate gels were stained using standard methods (Hebert and Beaton, 1993) for the following loci: amylase (AMY), mannose-6-phosphate isomerase (MPI), peptidase (PEP) using leucyl-glycine as a substrate, phosphoglucomutase (PGM), phosphoglucose isomerase (= glucose-6-phosphate isomerase, PGI). The phylogeny was constructed using CONTML and bootstrapped using SEQBOOT in PHYLIP 3.573c (Felsenstein, 1991).

Correlation between morphometric and molecular distances

To test correlation between genetic and morphometric distances, Mantel's test (Mantel, 1967) was performed using R-PACKAGE version 4.0 (Legendre & Vaudor, 1991). Correlations were determined **(1)** between mitochondrial DNA (mtDNA) and nuclear (allozyme) distances and **(2)** between mtDNA and morphometric distances. Pairwise maximum likelihood distances of mtDNA sequences were computed using DNADIST in PHYLIP version 3.573c (Felsenstein, 1991). The model of evolution employed a transition:transversion ratio (R) of 2.9, which was computed using maximum likelihood (DNAML). Euclidean distances were computed for canonical variates of morphometric measurements using the program SIMIL in R-Package 4.0. Cavalli-Sforza and Edwards' (1967) chord distances (shown in Appendix III) were computed for allozyme allele frequencies using GENDIST in PHYLIP 3.573c (Felsenstein, 1991).

Mantel's test was performed on eleven populations for the comparison between mtDNA and allozyme loci, and on twenty-one populations (shown in Fig. 5) for the comparison between mtDNA and morphometric distances. The tests performed 999 permutations. A partial Mantel test was also performed to remove effects of the European clade using a designer matrix (see R-Package documentation, www.fas.umontreal.ca/BIOL/Casgrain/en/labo/R/).

Interpopulation crosses

Data on interpopulation crosses presented here, %clutches yielding offspring that survived to adulthood, were taken from Table 2 in Lee (2000). Other measures of hybrid fitness are presented in Lee (2000), such as number of eggs per clutch, percentage survival per clutch, and development time. Interpopulation matings were performed between two genetically divergent clades (North Pacific and Atlantic+Gulf), between two genetically divergent subclades within the Atlantic+Gulf clade (Atlantic and North Atlantic), and within one subclade (Atlantic) (Table 1). The populations from divergent clades and subclades were chosen from regions where they come into contact (Table 1, Fig. 1) to determine whether genetically divergent but geographically proximate populations are reproductively isolated. Additionally, two populations within a subclade (Atlantic) from opposite coasts of the North American continent (sites 9 and 23) were crossed (Fig. 1), to determine whether speciation has occurred between genetically proximate but geographically distant populations. The population on the west coast (site 23) was probably a recent invasion resulting from the introduction of striped-bass from the east coast (Lee, 2000; Orsi, 2001).

Molecular versus quantitative genetic subdivision

Genetic subdivision of nuclear molecular markers (G_{ST}) (Nei, 1973) and quantitative (morphological) traits (Q_{ST}) (Spitze, 1993; Lynch et al., 1999) were compared in order to infer the relative roles of genetic drift and natural selection as causes of morphological divergence. Similar values for G_{ST} and Q_{ST} would suggest neutral evolution of morphological traits, without rejecting the possibility of selection (Lynch et al., 1999; Merilä & Crnokrak, 2001). A higher Q_{ST} value would suggest the presence of diversifying directional selection, while a lower value would suggest some type of constraining force such as stabilizing selection or lack of genetic diversity (Spitze, 1993; Lynch et al., 1999; Merilä & Crnokrak, 2001). G_{ST} of nuclear (allozyme) loci was computed using the program DISPAN (Genetic Distance and Phylogenetic Analysis) (Ota, 1993). Genetic subdivision of quantitative (morphological) traits (Q_{ST}) was measured using $Q_{ST} = \sigma^2_{GB} / (2\sigma^2_{GW} + \sigma^2_{GB})$, where σ^2_{GB} is the variance between populations and σ^2_{GW} is the mean additive genetic variance within populations (Spitze, 1993; Lynch et al., 1999). Variances within and among populations were calculated using standard ANOVA methods (p. 184 in (Sokal & Rohlf, 1995). Total morphometric variance within populations was multiplied by broad sense heritability for each character to obtain additive genetic variance within populations (σ^2_{GW}). Broad sense heritabilities for morphometric traits were obtained by computing intraclass correlations (Brodie & Garland, 1993; Dohm, 2002) for 8 full-sib clutches reared under common-garden

Table 1. Geographic and genetic distances between crossed populations of *E. affinis*. See Figure 3 for key to clade assignments (in circles)

Population Crosses (site #) (clade)		Geographic Distance (km)	%MtDNA Divergence		Nuclear Distance[a]
			16S	COI	
Waquoit Bay, MA (5) ⊗	x Edgartown Great Pond, MA (9) ●	20	5.16	10.6	0.1906
Edgartown Great Pond, MA (9) ●	x Grays Harbor marsh, WA (23) ●	4000[b]	0.96	0.15	0.1031
Grays Harbor marsh, WA (23) ●	x Columbia River estuary, OR (21) ○	55	7.66	17.1	0.3891

[a] Cavalli-Sforza & Edwards' (1967) chord distances based on five allozyme loci (see Appendix III).
[b] Grays Harbor salt marsh population is probably a recent cryptic invasion from the northeast coast of North America (Lee, 2000).

conditions (described below). The population from the St. Lawrence salt marsh (site 2) was used for heritability estimates.

G_{ST} was determined only for populations within North America (Table 2-1) because live or frozen (at – 80°C) tissue, required for allozyme electrophoresis, was not available from the Asian or European populations. Q_{ST} was calculated for four laboratory-reared populations from three North American clades or subclades (Appendix I), to reveal genetically-based patterns of morphological variation exclusive of environmental factors. The four laboratory populations were reared under common-garden conditions in a 13°C incubator at 15 PSU (parts per thousand salinity) for at least ten generations on a 15L:9D light cycle. The populations were fed their preferred food source of *Rhodomonas* sp. There was no evidence of founder effects in the populations, as the founding population sizes were in the hundreds and heterozygosities of the populations remained high (C. E. Lee, pers. obs.).

The Q_{ST} statistic was also computed for wild populations in order to estimate the effect of environment on morphology and to gain Q_{ST} estimates for populations that could not be reared in the laboratory. This Q_{ST} statistic for wild populations is technically not equivalent to quantitative genetic subdivision, because environmental factors could contribute to their phenotypic variance. The Q_{ST} statistic was computed for four wild populations (sites 5, 8, 11, 21) from the same North American clades and subclades as the lab-reared populations, 11 wild populations from North America (listed in Appendix III), 18 populations from all but the European clade, and 23 wild populations from all clades (Appendix II).

Results

Morphological versus molecular phylogenies

Molecular (Figs 3 and 4) and morphological (Fig. 5) phylogenies were not congruent, as evident from (1) morphological stasis despite dramatic genetic divergences among lineages, and (2) discordant patterns of morphological and molecular evolution. The morphological phylogeny revealed little divergence and no discernible hierarchical relationship among all Amerasian populations, which formed a polytomy (multifurcation) (Fig. 5A), while the molecular phylogenies showed considerable genetic structure and divergences separating these clades (Figs 3 and 4). Discordant patterns of morphological and molecular evolution were apparent when examining the European and North Pacific clades. The European clade alone was morphologically divergent from other clades (Fig. 5), but not especially divergent in the mtDNA phylogeny (Fig. 3). Also, within the European clade morphological divergences were large (Fig. 5A), while genetic divergences were small (Fig. 3). Conversely, the North Pacific clade was genetically the most divergent from all other clades (Fig. 3), but morphologically indistinguishable from other Amerasian clades (Fig. 5). The fact that the morphological phylogeny was constructed using wild-caught rather than lab-reared animals probably did not affect the tree-topology (Fig. 5), because mean values of traits used to construct the phylogeny were similar between lab and wild populations (Table 2-2, columns G, H; Appendices I, II). However, similarity between lab and wild populations, and possible effects of environmental plasticity on morphology, has not been determined for the European clade.

Mantel's test revealed a weak significant correlation between the morphological and mtDNA phylogenies (r = 0.266, p = 0.005, Mantel's t = 2.95). This significant correlation was biased, however, by the effect of the European clade, which was both ge-

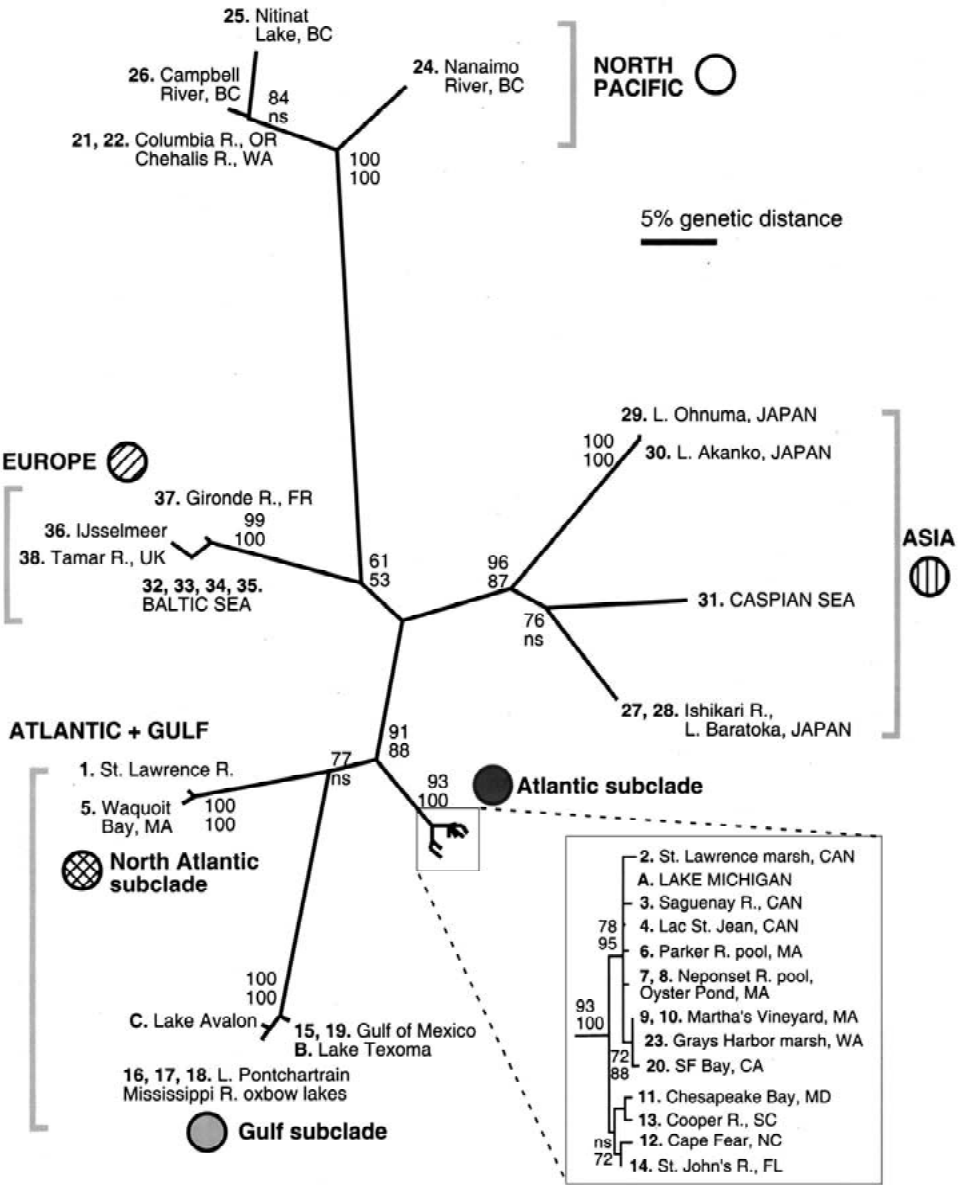

Figure 3. Unrooted phylogeny of *E. affinis* using the mitochondrial cytochrome oxidase I (COI, 652 base pairs). Populations are shown at branch tips, with numbers and letters referring to locations shown in Figure 1. Gray brackets (]) indicate the four major clades, and patterned circles represent distinct clades and subclades. The phylogeny was constructed with a distance matrix approach using PAUP* 4.0. Branch lengths reflect genetic distances, with scale bar indicating 5% genetic distance (maximum likelihood). Numbers next to nodes are bootstrap values based on 100 bootstrap replicates using distance matrix (upper number) and parsimony approaches (lower number) (Felsenstein, 1985). Bootstrap values of 'ns' indicate branches not supported by values greater than 50% for a given phylogenetic method. Adapted from Lee (2000).

netically and morphologically divergent from other populations, while the other populations were genetically, but not morphologically divergent. When a partial Mantel test was applied to remove effects of the European *versus* other clades, the correlation was extremely weak and non-significant (r= 0.0662, p = 0.230).

Nuclear versus mitochondrial phylogenies

The nuclear phylogeny (Fig. 4) was congruent with the mtDNA phylogeny (Fig. 3), suggesting that both the mtDNA and allozyme loci conform to neutral expectations. Mantel's test revealed a positive significant correlation between the phylogenies (r = 0.680, p =

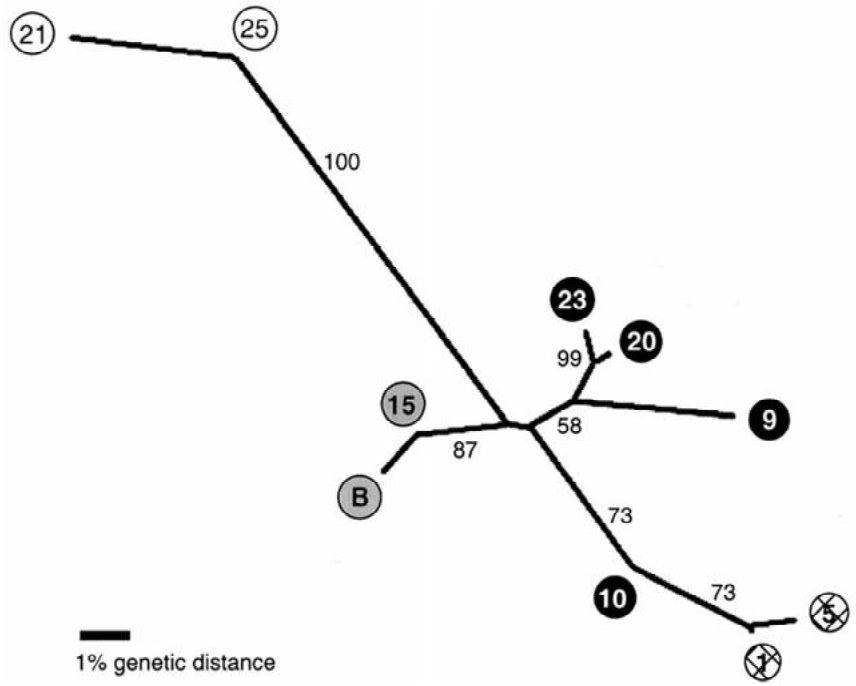

Figure 4. Nuclear phylogeny based on five allozyme loci. Branch lengths are Cavalli-Sforza & Edwards' (1967) chord distances. Phylogeny was constructed using CONTML in PHYLIP version 3.573c (Felsenstein, 1991). Numbers on branches are bootstrap values for 100 replicates. Patterned circles represent clades shown in Figure 3, and numbers and letters within the circles represent populations shown in Figures 1 and 3.

0.001, Mantel's t = 3.96). An exception to the concordance was the clustering of the Tisbury Great Pond, Martha's Vineyard population (site 10) with the North Atlantic clade in the allozyme phylogeny (Fig. 4). In the mtDNA phylogeny (Fig. 3) the Tisbury population clustered with the Atlantic clade. This pattern could be evidence of past introgression between the two clades, despite the fact that the clades currently appear to be reproductively isolated (Fig. 6). Alternatively, the clustering of the Tisbury population with the North Atlantic clade could have resulted from cryptic differentiation of alleles. For instance, Tisbury shared the same allele with the North Atlantic clade at the amylase (AMY) locus, which is nearly fixed for different clades. It is possible that those bands which shared mobilities actually differed in amino acid or DNA sequence.

Morphological and molecular evolution versus reproductive isolation

Patterns of morphological (Fig. 5) and molecular evolution (Figs 3, 4) were discordant with patterns of reproductive isolation, shown here as percentage of clutches with offspring that survived to adulthood (Fig. 6). For instance, two clades within the North American continent (North Pacific and Atlantic+Gulf) were morphologically close (Fig. 5A) but genetically most divergent (19% in COI; Fig. 3) and reproductively isolated (Fig. 6A). Most notably, populations within a single clade (sites 9 and 23; Atlantic) exhibited little morphological (Fig. 5A) or genetic (Figs 3 and 4) divergence, yet were reproductively isolated (Fig. 6B). Thus, at least for some cases, reproductive isolation proved to be the most sensitive measure of speciation for *E. affinis*.

Genetic subdivision of nuclear molecular and morphological characters

Molecular genetic subdivision was high for four populations from North America (G_{ST} = 0.617), while these same populations reared in the laboratory exhibited low levels of morphological subdivision (mean Q_{ST} across all characters = 0.162). The high G_{ST} indicated that the majority of the molecular genetic variance was distributed among populations, while the low Q_{ST} revealed retarded rates of morphological evolu-

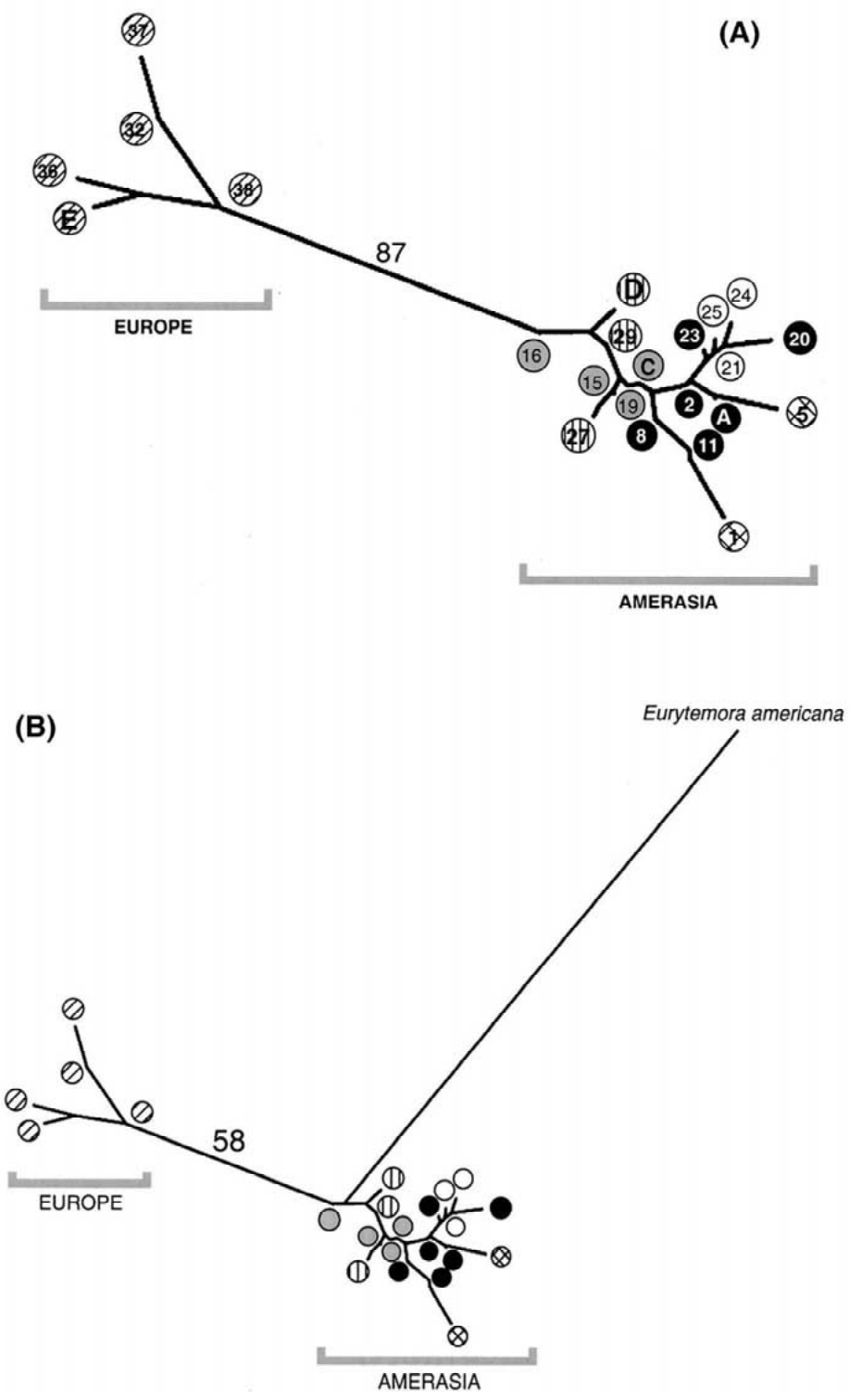

Figure 5. Morphological Phylogeny of **(A)** *E. affinis* complex and **(B)** *E. affinis* complex with congener *E. americana* as an outgroup species. Phylogenies were constructed using CONTML in PHYLIP version 3.573c (Felsenstein, 1991). Number on branch represents a bootstrap value based on 100 replicates. Other nodes were not statistically supported. Patterned circles represent the clades shown in Figure 3. Numbers within the circles represent populations shown in Figure 3, except for two populations not included in the molecular phylogenies: D = Black Sea, and E = Hickling Broad, Norfolk, U.K.

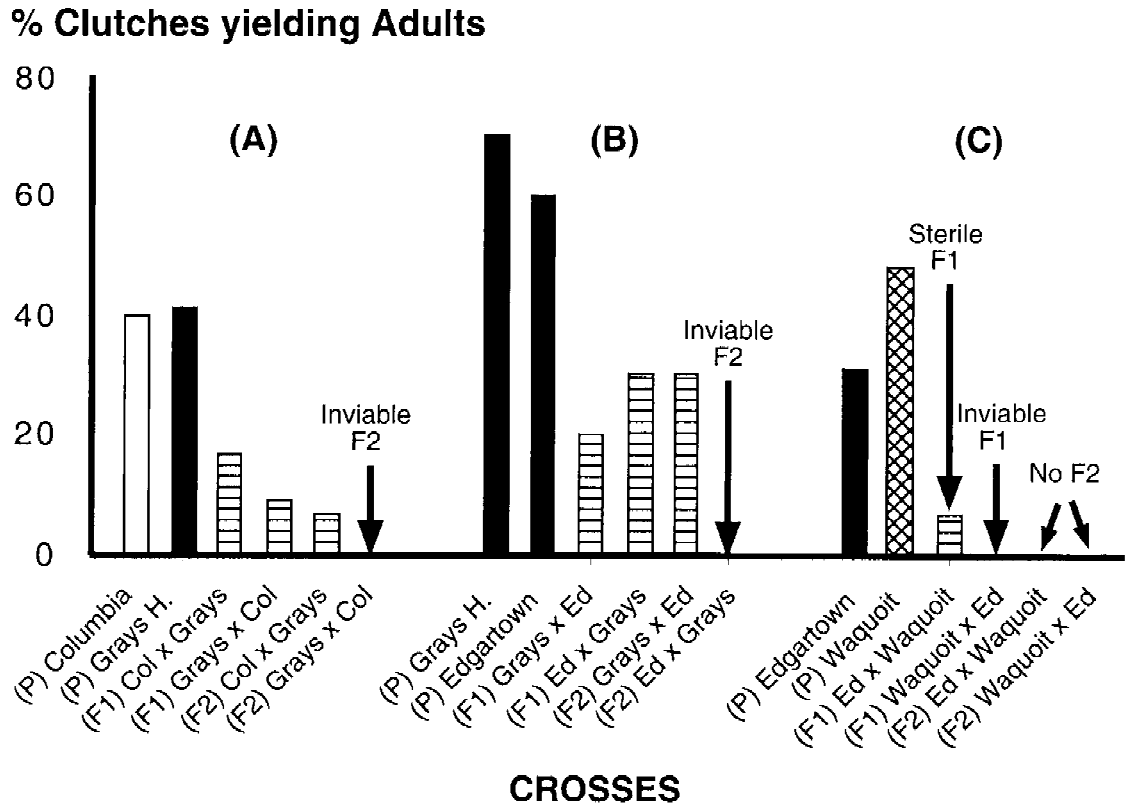

Figure 6. Hybrid breakdown of interpopulation crosses (described in Table 1). Vertical axis shows percentage of clutches containing progeny that developed to adulthood. For each group of bars (**A–C**), the first two bars represent survival of F1 progency resulting from crosses within each parental population (**P**), with patterns of those bars referring to the clades shown in Figure 2. The remaining bars (with horizontal hatching) represent survival of F1 and F2 offspring from each reciprocal cross **(A)** between two clades (North pacific and North America), **(B)** within a clade (Atlantic), and **(C)** between two subclades (Atlantic and North Atlantic). Adapted from Lee (2000).

tion among these populations (Tables 2-1, 2-2). The Q_{ST} statistic computed for four wild populations (Q_{ST} = 0.292) was higher than for laboratory-reared populations, suggesting that environmental factors could have affected phenotypic values in the wild (Table 2-1, columns A and B). Thus, secondary sex characters could have a plastic response to environmental conditions, though the effect is not large. For wild populations, Q_{ST} values remained lower than G_{ST} (Table 2-1, columns B and C). Not surprisingly, mean Q_{ST} increased with the inclusion of additional clades, particularly Europe, because of increases in interpopulation variance (Table 2-1, columns D and E).

Discussion

Morphological stasis

Ernst Mayr recognized that morphological evolution and speciation might be discordant in his discussion of sibling species (Mayr, 1963). This study revealed stark discordances among rates of molecular evolution (Figs 3 and 4), morphological divergence (Fig. 5), and reproductive isolation (Fig. 6). For instance, reproductive isolation between genetically proximate and morphologically indistinguishable populations (Fig. 6) indicated that morphological stasis does not reflect evolutionary stasis, but cryptic speciation (see Introduction). Morphological stasis within the Amerasian clades was evident from**(1)** the morphological phylogeny (Fig. 5A), which revealed an unresolved polytomy (multifurcation) of short branches among Amerasian clades, in contrast to large genetic diver-

gences (Figs 3, 4) and **(2)** the low level of morphological subdivision (Q_{ST} = 0.162) for laboratory-reared populations, accompanied by higher levels of molecular genetic subdivision (G_{ST} = 0.617). The degree of persistence of morphological stasis is difficult to determine without a molecular clock for *E. affinis*. Interpopulation sequence divergences of up to 19% in COI and rate calibrations of molecular evolution for other crustaceans (1.4 – 2.3%/my for COI) (Knowlton & Weigt, 1998; Schubart et al., 1998) suggest that morphological stasis within the Amerasian clades has persisted for 8 to 14 million years.

The morphological stasis evident in the Amerasian clade might not apply for the European clade. The morphological phylogeny (Fig. 5A) revealed large morphological divergence between the European clade and other clades, as well as within this clade. Also, morphological subdivision of wild populations increased when this clade was included (Table 2-1, column E). It is possible that Q_{ST} would be much higher than G_{ST} with the inclusion of Europe, suggesting directional selection on the morphology of this clade alone. However, wild populations were used for both the morphological phylogeny and for the Q_{ST} value including Europe (Table 2-1, column E), such that possibility of environmental plasticity could not be excluded. Lab-reared populations from Europe would be required for an accurate estimation of morphological divergence devoid of environmental effects.

Species concepts for the Eurytemora affinis *complex*

Species boundaries cannot be defined clearly and species concepts are problematic when applied to this species complex. The difficulty in defining species boundaries reflects the complex and idiosyncratic manner by which speciation occurs. The Morphological Species Concept is clearly not feasible for *E. affinis* because morphological (secondary sex) characters divide this species complex into two groups (Fig. 5, Europe and Amerasia), ignoring most of the large genetic distances within the complex. Using general morphology (body size, shape, or proportions) would lead to spurious divisions due to environmental plasticity, as in the case of the erroneous subdivision of *E. affinis* into invalid species, such as *E. hirundo* and *E. hirundoides* (Busch & Brenning, 1992; Lee, 2000). The Phylogenetic Species Concept (Cracraft, 1983) is problematic because of the inability to distinguish between genetically proximate populations that are reproductively isolated (sites 9 and 23, Table 1, Fig. 6). Also, there is the possibility of introgression between two genetically distant clades (Atlantic and North Atlantic, Figs 3 and 4), such that monophyletic units might vary among genetic markers. The Biological Species Concept (Dobzhansky, 1937) poses problems because asymmetries in reproductive isolation and varying degrees of hybrid viability make the boundaries difficult to establish (Fig. 6). However, reproductive isolation does appear to be a more sensitive indicator of speciation than the molecular markers used in this study (compare Figs 3 and 6). Inconsistencies among different measures of evolutionary divergence emphasize the importance of using a multifaceted approach toward the study of speciation and species boundaries (Knowlton, 1993).

Evolutionary forces underlying morphological stasis

The lack of morphological differentiation among all but the European clade was remarkable (Fig. 5). Given that gene flow appeared to be absent among most populations (Lee, 2000), it is surprising that morphological divergence did not arise from either selection or genetic drift. One would expect both sexual selection and natural selection to operate on the secondary sex characters examined in this study. For morphological stasis to arise, constraining forces must be greater than the diversifying effects of selection or drift.

Comparisons between nuclear molecular genetic (G_{ST}) and quantitative genetic (Q_{ST}) subdivision could yield insights into evolutionary forces driving morphological differentiation (Spitze, 1993; Lynch et al., 1999; Merilä and Crnokrak, 2001). Lower values for Q_{ST} (0.162) for laboratory-reared populations relative to G_{ST} (0.617) suggest retarded evolution of secondary sex characters, possibly as a result of stabilizing (Lynch & Spitze, 1994; Lynch et al., 1999) or fluctuating selection (Kawecki, 2000). Unlike results of this study, for most species Q_{ST} typically exceeds G_{ST} or F_{ST}, suggesting the action of directional selection on morphological characters (Merilä & Crnokrak, 2001). Thus, evidence for retarded morphological evolution has been relatively rare (Merilä & Crnokrak, 2001).

Diversifying effects of sexual selection on morphological traits might be weak for *E. affinis*, in contrast to other species where the strength of sexual selection greatly exceeds viability selection based on survival (Hoekstra et al., 2001). For *E. affinis*, opportunities for

Table 2-1. Q_{ST} and G_{ST} values for laboratory-reared and wild populations and morphological variances among and within populations. Q_{ST} was calculated for (A) four laboratory-reared populations from North America (Appendix I), (B) four wild populations from North America, (C) all North American clades and subclades, (D) all clades except for Europe, and (E) all clades. N = number of populations

	(A) Lab-Reared $N = 4$	(B) Wild $N = 4$	(C) Wild $N = 11$	(D) Wild $N = 18$	(E) Wild $N = 23$ (all clades)
G_{ST} based on five allozyme locis	0.617	0.559	0.547		
MEAN Q_{ST} across all characters	0.162	0.292	0.330	0.453	0.817
MEAN additive genetic variance among populations (σ^2_{GB})	0.00115	0.00181	0.000811	0.00256	0.0245
MEAN additive genetic variance within populations (σ^2_{GW})	0.00190	0.00236	0.00309	0.00277	0.00246

Table 2-2. Q_{ST} values (columns A–E) and broad sense heritabilities (column F) for eight morphometric characters, and comparison of means between laboratory-reared and wild populations (columns G, H). Means of morphometric characters were compared between lab and wild populations from the Columbia River estuary (site 21) and Waquoit Bay (site 5) using a Student's t-test (columns G, H)

		Q_{ST}					h^2	Student's t (p-value)	
		(A) Lab-Reared $N = 4$	(B) Wild $N = 4$	(C) Wild $N = 11$	(D) Wild $N = 18$	(E) Wild $N = 23$	(F) Full-sib	(G) Lab *vs.* Wild Columbia R. (21)	(H) Lab *vs.* Wild Waquoit Bay (5)
Morphological Character									
FEMALE	GSW/PL (genital segment width)	0.158	0.420	0.432	0.476	0.790	0.995	2.63 (0.025)	2.83 (0.022)
	GSW/GSL (genital segment shape)	0.00296	0.494	0.269	0.503	0.833	0.353	0.29 (0.77)	2.74 (0.026)
	A1 24:22 (antennule proportions)	0.0641	0.135	0.387	0.338	0.844	0.899	0.22 (0.83)	−1.30 (0.23)
MALE	rtP5 exo1/PL (5th leg, right exopod 1)	0.202	0.583	0.444	0.632	0.810	1.000	−0.72 (0.49)	−1.26 (0.24)
	rtP5 exo2/PL (5th leg, right exopod 2)	0.188	0.328	0.285	0.372	0.713	1.000	−0.21 (0.84)	0.54 (0.61)
	lftP5 Bp2W/PL (5th leg, left basipod 2)	−0.283	−0.365	0.525	0.626	0.819	0.205	1.13 (0.29)	0.81 (0.44)
	rtP5 exo1/lft P5Bp2W (5th leg proportions)	0.248	0.0643	−0.0858	0.142	0.810	0.380	−1.32 (0.22)	−1.43 (0.19)
	A1 24:22 (antennule proportions)	0.713	0.678	0.382	0.536	0.916	0.254	0.54 (0.61)	0.87 (0.41)

morphologically-based sexual selection might be limited by the absence of visual cues for mating and lack of female choice (Katona, 1973; Doall et al., 1998; Weissburg et al., 1998; Yen et al., 1998). Without an image-forming eye, *E. affinis* uses olfactory rather than visual cues for mating (Katona, 1973). In many copepods, males pursue females by following their scent trails, and then force copulation upon the females (Doall et al., 1998; Weissburg et al., 1998; Yen et al., 1998). Thus, sexual selection in *E. affinis* might be manifested in the evolution of chemical signaling, rather than in morphological evolution of secondary sex characters. In addition to weak sexual selection on morphology, directional selection could be damped by overlapping generations induced by the presence of a diapausing egg bank (Ellner & Hairston, 1994), which could remain viable in the sediment for hundreds of years (Hairston, 1996).

Even if differentiating forces are absent or weak, constraining forces must be operating to prevent morphological divergence due to genetic drift. Support for stabilizing selection as a cause for morphological stasis was found in a quantitative genetic analysis of cheilostome bryozoans (Cheetham et al., 1994, 1995). Fluctuating selection might be viewed as equivalent to stabilizing selection (Charlesworth et al., 1982) with a shifting optimum over time. Under fluctuating selection, selection within a generation might be directional, but across multiple generations reversals in the direction of selection on a quantitative trait would produce indirect selection for canalization (Kawecki, 2000). This pattern of selection could ultimately lead

to developmental constraint or entrapment on a local fitness peak (Waddington, 1956; Gould, 1980; Nijhout & Emlen, 1998).

Such a fluctuating selection regime (Kawecki, 2000) is likely to be imposed by fluctuating environments (Sheldon, 1996; Chiba, 1998). Fluctuating environments have been argued to promote the evolution of stasis, selecting for long-term generalists that are relatively inert to environmental changes (Sheldon, 1996; Chiba, 1998). Habitats of *E. affinis* are characterized by fluctuation in salinity, temperature and other variables over many times scales, including diel, seasonal, annual, and geological time scales. The coastal habitats in which *E. affinis* resides are extremely dynamic, with boundaries between estuaries, salt marshes, and coastal lakes under constant flux. A generalist strategy in response to fluctuating environments (Sheldon, 1996; Chiba, 1998) might be precluding populations of *E. affinis* from experiencing adaptive radiations in the wild. Yet, morphological divergence of the European clade remains unexplained, given that the European populations occur in similar types of environments as the other clades.

The comparison between Q_{ST} for laboratory-reared (0.162) and wild populations (0.292) could yield clues regarding selective pressures operating in the wild. Levels of additive genetic variance within laboratory populations (Table 2-1, column A; Appendix I) are not sufficiently low for lack of genetic diversity to be the cause of morphological stasis. On the other hand, levels of intrapopulation variance within wild populations (Table 2-1, columns B–E; Appendix II) are not adequately high to support the hypotheses that balancing selection or microheterogeneity within habitats resulted in the coexistence of diverse phenotypes, resulting in no net morphological change (Lieberman & Dudgeon, 1996). Higher Q_{ST} values for wild relative to laboratory populations (Table 2) suggests that plasticity has affected morphology of secondary sex characters in the wild, and might have buffered against evolutionary change (Wake et al., 1983), but the effect is not large.

On the other hand, plasticity might play a more important role in buffering evolutionary change of general morphological features (such as body size, shape, proportions, etc.). For example, size differences occur in wild populations according to habitat type, with larger adults in salt marshes and lakes (prosome length: 909.5 μm $\pm$ 33.4 SE, $N = 14$ populations), and smaller adults in estuaries (prosome length: 770.4 μm $\pm$ 26.5 SE, $N = 20$ populations). These size differences disappear in the laboratory under constant conditions (C. E. Lee, unpublished data). Thus, forces responsible for stasis might differ between secondary sex and general morphological characters.

In summary, this study revealed morphological stasis in secondary sex characters in all clades but Europe, and discrepancies among rates of morphological differentiation, molecular evolution, and reproductive isolation. The pattern of quantitative genetic (morphological) and molecular subdivision (i.e. lower Q_{ST} than G_{ST}) suggests that morphological stasis might be caused by stabilizing or fluctuating selection. In addition, diversifying sexual selection on morphological characters might be weak, given the absence of visual cues for mating and lack of female choice. Morphological stasis in secondary sex characters might be common in non-visual invertebrates, such as those residing in turbid aquatic systems or soils, due to a lack of sexual selection on morphology. Although not comprehensively tested, morphological stasis is probably common in free-living copepods (Frost, 1974; Frost, 1989; Sevigny et al., 1989; McKinnon et al., 1992; Bucklin et al., 1995; Burton, 1998), and other aquatic microcrustaceans, such as *Daphnia* (Colbourne & Hebert, 1996). Our assumptions regarding rapid evolution in secondary sex characters might be biased by the preponderance of studies on terrestrial organisms with conspicuous polymorphisms. Thus, cryptic speciation might be far more common than previously assumed.

Acknowledgements

This paper was included in this special symposium volume in honor of Dr Bruce W. Frost's sixtieth birthday. This project was funded by NSF DEB-0130543 to C. E. Lee. Greg Gelembiuk and James Crow provided thoughtful discussion and members of C. E. Lee's laboratory provided useful editorial comments. Greg Gelembiuk assisted with computation of canonical variates and Q_{ST}. This paper was improved by insightful comments from an anonymous reviewer. For samples not included in Lee (1999) or Lee (2000), Ken Irvine collected samples from Hickling Broad, UK, and Mark Ohman provided samples from the Black Sea.

References

Brodie, E. D. I. & T. J. Garland, 1993. Quantitative genetics of snake populations. In Seigel, R. A. & J. T. Collins (eds), Snakes: Ecology and Behavior. McGraw Hill, New York: 315–362

Bucklin, A., B. W. Frost & T. D. Kocher, 1995. Molecular systematics of six *Calanus* and three *Metridia* species (Calanoida: Copepoda). Mar. Biol. 121: 655–664.

Burton, R. S., 1990. Hybrid breakdown in developmental time in the copepod *Tigriopus californicus*. Evolution 44: 1814–1822.

Burton, R. S., 1998. Intraspecific phylogeography across the Point Conception biogeographic boundary. Evolution 52: 734–745.

Busch, A. & U. Brenning, 1992. Studies on the status of *Eurytemora affinis* (Poppe, 1880) (Copepoda, Calanoida). Crustaceana 62: 13–38.

Campbell, N. A. & W. R. Atchley, 1981. The geometry of canonical variate analysis. Syst. Zool. 30: 268–280.

Cavalli-Sforza, L. L. & A. F. W. Edwards, 1967. Phylogenetic analysis and estimation procedures. Evolution 21: 550–570.

Charlesworth, B., R. Lande & M. Slatkin, 1982. A neo-Darwinian commentary on Macroevolution. Evolution 36: 474–498.

Cheetham, A. H., J. B. C. Jackson & L.-A. C. Hayek, 1995. Quantitative genetics of bryozoan phenotypic evolution: III. Phenotypic plasticity and the maintenance of genetic variation. Evolution 49: 290–296.

Cheetham, A. H., J. B. C. Jackson & L. A. C. Hayek, 1994. Quantitative genetics of bryozoan phenotypic evolution: II. Analysis of selection and random change in fossil species using reconstructed genetic parameters. Evolution 48: 360–375.

Chiba, S., 1998. A mathematical model for long-term patterns of evolution: Effects of environmental stability and instability on macroevolutionary patterns and mass extinctions. Paleobiology 24: 336–348.

Colbourne, J. K. & P. D. N. Hebert, 1996. The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. Phil. Trans. R. Soc. Lond. B 351: 349–360.

Cracraft, J., 1983. Species concepts and speciation analysis. In Johnston, R. F. (ed.), Current Ornithology. Plenum Press, New York: 159–187

Darwin, C., 1872. The Origin of Species, 6th Edition. John Murray, London.

Doall, M. H., S. P. Colin, J. R. Strickler & J. Yen, 1998. Locating a mate in 3D: The case of *Temora longicornis*. Phil. Trans. Royal. Soc. Lond. B 353: 681–689.

Dobzhansky, T., 1937. Genetics and the Origin of Species. Columbia University Press, New York.

Dohm, M. R., 2002. Repeatability estimates do not always set an upper limit to heritability. Funct. Ecol. 16: 273–280.

Edmands, S., 1999. Heterosis and outbreeding depression in interpopulation crosses spanning a wide range of divergence. Evolution 53: 1757–1768.

Ellner, S. & N. G. Hairston, 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. Am. Nat. 143: 403–417.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

Felsenstein, J., 1988. Phylogenies and quantitative characters. Ann. Rev. Ecol. Syst. 19: 445–471.

Felsenstein, J., 1991. PHYLIP: Phylogeny Inference Package (version 3.5). Dept. of Genetics, Univ. of Washington, Seattle, WA.

Frost, B. W., 1974. *Calanus marshallae*, a new species of calanoid copepod closely allied to the sibling species *C. finmarchicus* and *C. glacialis*. Mar. Biol. 26: 77–99.

Frost, B. W., 1989. A taxonomy of the marine calanoid copepod genus *Pseudocalanus*. Can. J. Zool. 67: 525–551.

Ganz, H. H. & R. S. Burton, 1995. Genetic differentiation and reproductive incompatibility among Baja California populations of the copepod *Tigriopus californicus*. Mar. Biol. 123: 821–827.

Gould, S. J., 1980. Is a new and general theory of evolution emerging? Paleobiology 6: 119–130.

Gould, S. J., 1982. The meaning of punctuated equilibrium and its role in validating a hierarchical approach to macroevolution *in* R. Milkman, ed., Perspectives on Evolution. Sinauer Associates, Inc., Sunderland, MA.

Gould, S. J. & N. Eldredge, 1977. Punctuated equilibria: the tempo and mode of evolution reconsidered. Paleobiology 3: 115–151.

Hairston, N. G. J., 1996. Zooplankton egg banks as biotic reservoirs in changing environments. Limnol. Oceanogr. 41: 1087–1092.

Hebert, P. D. N. & M. J. Beaton, 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories, Beaumont, TX.

Hoekstra, H. E., J. M. Hoekstra, D. Berrigan, S. N. Vignieri, A. Hoang, C. E. Hill, P. Beerli & J. G. Kingsolver, 2001. Strength and tempo of directional selection in the wild. Proc. Natl. Acad. Sci. USA 98: 9157–9160.

Hoelzel, A. R. & A. Green, 1992. Analysis of population-level variation by sequencing PCR-amplified DNA. In Hoelzel, A. R. (ed.), Molecular Genetic Analysis of Populations: A Practical Approach. Oxford University Press, New York: 159–187.

Jackson, J. B. C. & A. H. Cheetham, 1990. Evolutionary significance of morphospecies: A test with cheilostome Bryozoa. Science 248: 579–583.

Jackson, J. B. C. & A. H. Cheetham, 1994. Phylogeny reconstruction and the tempo of speciation in cheilostome bryozoa. Paleobiology 20: 407–423.

Katona, S. K., 1973. Evidence for sex pheromones in planktonic copepods. Limnol. Oceanogr. 18: 574–583.

Kawecki, T. J., 2000. The evolution of genetic canalization under fluctuating selection. Evolution 54: 1–12.

King, J. L. & R. Hanner, 1998. Cryptic species in a 'living fossil' lineage: Taxonomic and phylogenetic relationships within the genus *Lepidurus* (Crustacea: Notostraca) in North America. Mol. Phyl. Evol. 10: 23–36.

Knowlton, N., 1993. Sibling species in the sea. Annu. Rev. Ecol. Syst. 24: 189–216.

Knowlton, N., 2000. Molecular genetic analyses of species boundaries in the sea. Hydrobiologia 420: 73–90.

Knowlton, N. & L. A. Weigt, 1998. New dates and new rates for divergence across the isthmus of Panama. Proc. R. Soc. Lond. B 265: 2257–2263.

Lee, C. E., 1999. Rapid and repeated invasions of fresh water by the saltwater copepod *Eurytemora affinis*. Evolution 53: 1423–1434.

Lee, C. E., 2000. Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate 'populations'. Evolution 54: 2014–2027.

Lee, C. E., 2002. Evolutionary genetics of invasive species. Trends Ecol. Evol. 17: 386–391.

Lee, C. E. & M. A. Bell, 1999. Causes and consequences of recent freshwater invasions by saltwater animals. Trends Ecol. Evol. 14: 284–288.

Lee, C. E. & C. H. Petersen, 2002. Genotype-by-environment interaction for salinity tolerance in the freshwater invading copepod *Eurytemora affinis*. Phys. Biochem. Zool. 75: 335–344.

Legendre, P. & A. Vaudor, 1991. The R Package: Multidimensional Analysis, Spatial Analysis. University of Montreal, Montreal.

Lieberman, B. S. & S. Dudgeon, 1996. An evaluation of stabilizing selection as a mechanism for stasis. Palaeogeogr. Palaeoclimatol. Palaeoecol. 127: 229–238.

Lynch, M., M. Pfrender, K. Spitze, N. Lehman, J. Hicks, D. Allen, L. Latta, M. Ottene, F. Bogue & J. Colbourne, 1999. The quantitative and molecular genetic architecture of a subdivided species. Evolution 53: 100–110.

Lynch, M. & K. Spitze, 1994. Evolutionary genetics of *Daphnia*. In Real, L. A. (ed.), Ecological Genetics. Princeton University Press, Princeton: 109–128.

Mantel, N., 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209–220.

Mayr, E., 1963. Animal Species and Evolution. Harvard University Press, Cambridge.

McKinnon, A. D., W. J. Kimmerer & J. A. H. Benzie, 1992. Sympatric sibling species within the genus *Acartia* (Copepoda: Calanoida): a case study from Westernport and Port Phillip Bays, Australia. J. Crust. Biol. 12: 239–259.

Merilä, J. & P. Crnokrak, 2001. Comparison of genetic differentiation at marker loci and quantitative traits. J. Evol. Biol. 14: 892–903.

Müller, J., E. Partsch & A. Link, 2000. Differentiation in morphology and habitat partitioning of genetically characterized *Gammarus fossarum* forms (Amphipoda) across a contact zone. Biol. J. Linnean Soc. 69: 41–53.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Nat. Acad. Sci. USA 70: 3321–3323.

Nijhout, H. F. & D. J. Emlen, 1998. Competition among body parts in the development and evolution of insect morphology. Proc. Nat. Acad. Sci. USA 95: 3685–3689.

Orsi, J., 2001. *Eurytemora affinis* is introduced. IEP Newsletter 14: 12.

Ota, T., 1993. DISPAN: Genetic Distance and Phylogenetic Analysis. Institute of Molecular Evolutionary Genetics, Pennsylvania State University.

Palmer, A. R., S. D. Gayron & D. S. Woodruff, 1990. Reproductive, morphological, and genetic evidence for two cryptic species of northeastern Pacific *Nucella*. Veliger 33: 325–338.

Poppe, S. A., 1880. Über eine neue Art der Calaniden-Gattung *Temora*, Baird. Abhandlg. Naturw. Verein Bremen 7: 55–60.

Rempe, U. & E. E. Weber, 1972. An illustration of the principal ideas of MANOVA. Biometrics 28: 235–238.

Schmeil, O., 1896. Deutschlands freilebende Süsswasser-Copepoden. Erwin Nägele, Stuttgart.

Schubart, C. D., R. Diesel & S. B. Hedges, 1998. Rapid evolution to terrestrial life in Jamaican crabs. Nature 393: 363–365.

Sevigny, J.-M., I. A. Mclaren & B. W. Frost, 1989. Discrimination among and variation within species of *Pseudocalanus* based on the GPI locus. Mar. Biol. 102: 321–328.

Sheldon, P. R., 1996. Plus ça change – a model for stasis and evolution in different environments. Palaeogeogr. Palaeoclimatol. Palaeoecol. 127: 209–227.

Simpson, G. G., 1944. Tempo and Mode in Evolution. Columbia University Press, New York.

Sokal, R. R. & F. J. Rohlf, 1995. Biometry. W. H. Freeman and Company, New York.

Spitze, K., 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. Genetics 135: 367–374.

Taylor, D. J., P. D. N. Hebert & J. K. Colbourne, 1996. Phylogenetics and evolution of the *Daphnia longispina* group (Crustacea) based on 12S rDNA sequence and allozyme variation. Mol. Phyl. Evol. 5: 495–510.

Waddington, C. H., 1956. Principles of Embryology. George Allen & Unwin., London.

Wake, D. B., G. Roth & M. H. Wake, 1983. On the problem of stasis in organismal evolution. J. Theor. Biol. 101: 211–224.

Weissburg, M. J., M. H. Doall & J. Yen, 1998. Following the invisible trail: Kinematic analysis of mate-tracking in the copepod *Temora longicornis*. Phil. Trans. Royal. Soc. Lond. B 353: 701–712.

Yen, J., M. J. Weissburg & M. H. Doall, 1998. The fluid physics of signal perception by mate-tracking copepods. Phil. Trans. Royal. Soc. Lond. B 353: 787–804.

Appendix I. Morphometric measurements of laboratory-reared populations, four North American populations of *E. affinis* and one population of *E. americana*. Values are means for seven replicates with standard deviations in parentheses. Abbreviations for morphological characters are described in 'Methods and Materials'

CLADE /SUBCLADE	(site#) LOCATION	FEMALE			MALE				
		GSW/ PL	GSW/ GSL	A1 24:22	rtP5exo1/ PL	rtP5exo2/ PL	lftP5Bp2W/ PL	rtP5exo1/ lftP5Bp2W	A1 24:22
North Atlantic									
	(5) Waquoit Bay	0.240	1.739	1.326	0.231	0.226	0.0927	2.495	1.362
		(0.016)	(0.108)	(0.0873)	(0.0080)	(0.0073)	(0.0043)	(0.122)	(0.043)
Atlantic									
	(9) Edgartown Pond	0.227	1.773	1.400	0.234	0.226	0.0909	2.580	1.377
		(0.0076)	(0.083)	(0.0641)	(0.0051)	(0.0056)	(0.0032)	(0.131)	(0.054)
	(13) Cooper River	0.238	1.744	1.360	0.243	0.236	0.0924	2.639	1.362
		(0.0088)	(0.103)	(0.0431)	(0.0106)	(0.0104)	(0.0055)	(0.111)	(0.095)
North Pacific									
	(21) Columbia River	0.246	1.674	1.324	0.230	0.239	0.0933	2.473	1.226
		(0.0091)	(0.138)	(0.0742)	(0.0039)	(0.0106)	(0.0045)	(0.113)	(0.057)
E. americana									
	Duwamish River, WA	0.172	1.099	1.342	0.204	0.230	0.0562	3.643	1.338
		(0.0058)	(0.0454)	(0.079)	(0.0117)	(0.0103)	(0.0036)	(0.202)	(0.050)

Appendix II. Morphometric measurements of 23 wild-caught populations from all clades within the *Eurytemora affinis* complex. The morphological phylogeny in Figure5 was constructed using these values. Values are means for three to five replicates with standard deviations in parentheses. Abbreviations for morphological characters are described in 'Methods and Materials'

CLADE /SUBCLADE	(site#) LOCATION	FEMALE			MALE				
		GSW/ PL	GSW/ GSL	A1 24:22	rtP5exo1/ PL	rtP5exo2/ PL	lftP5Bp2W/ PL	rtP5exo1/ lftP5Bp2W	A1 24:22
North Atlantic									
	(1) St. Lawrence River	0.183 (0.0151)	1.558 (0.0909)	1.484 (0.0304)	0.216 (0.0054)	0.211 (0.0184)	0.0887 (0.0054)	2.446 (0.165)	1.345 (0.0825)
	(5) Waquoit Bay	0.212 (0.0074)	1.557 (0.0519)	1.406 (0.0942)	0.239 (0.0116)	0.2226 (0.0152)	0.0903 (0.0041)	2.650 (0.233)	1.3357 (0.0440)
Atlantic									
	(2) St. Lawrence marsh	0.242 (0.0184)	1.803 (0.2104)	1.296 (0.0341)	0.227 (0.0079)	0.226 (0.0044)	0.0875 (0.0043)	2.599 (0.0730)	1.343 (0.0481)
	(8) Oyster Pond	0.221 (0.0040)	1.766 (0.0274)	1.372 (0.0526)	0.218 (0.0080)	0.220 (0.0057)	0.0886 (0.0088)	2.473 (0.154)	1.379 (0.0622)
	(11) Chesapeake Bay	0.204 (0.0197)	1.768 (0.151)	1.384 (0.0421)	0.205 (0.0043)	0.211 (0.0074)	0.0848 (0.0039)	2.418 (0.0990)	1.363 (0.0677)
	(A) Lake Michigan	0.238 (0.0165)	1.778 (0.130)	1.405 (0.0248)	0.231 (0.0102)	0.230 (0.0172)	0.0951 (0.0022)	2.424 (0.114)	1.377 (0.0766)
	(20) San Francisco Bay	0.242 (0.0225)	1.711 (0.188)	1.328 (0.0857)	0.248 (0.0154)	0.255 (0.0123)	0.0984 (0.0003)	2.519 (0.158)	1.326 (0.114)
	(23) Grays H. marsh	0.230 (0.0020)	1.789 (0.0864)	1.284 (0.0269)	0.233 (0.0009)	0.241 (0.0026)	0.0900 (0.0038)	2.587 (0.110)	1.257 (0.101)
Gulf									
	(15) Mississippi estuary	0.227 (0.0029)	1.578 (0.0437)	1.346 (0.0235)	0.216 (0.0072)	0.232 (0.0044)	0.0870 (0.0040)	2.485 (0.194)	1.382 (0.0703)
	(16) Lake Pontchartrain	0.209 (0.0041)	1.505 (0.0811)	1.423 (0.0312)	0.193 (0.0033)	0.207 (0.0067)	0.0826 (0.0037)	2.338 (0.0771)	1.451 (0.0551)
	(19) Colorado River	0.230 (0.0168)	1.729 (0.154)	1.317 (0.0304)	0.208 (0.0064)	0.224 (0.0094)	0.0866 (0.0001)	2.400 (0.0721)	1.348 (0.0721)
	(C) Lake Avalon	0.227 (0.0126)	1.717 (0.168)	1.268 (0.0659)	0.211 (0.0071)	0.218 (0.0075)	0.086 (0.0025)	2.444 (0.126)	1.356 (0.0299)
North Pacific									
	(21) Columbia River	0.234 (0.0065)	1.653 (0.102)	1.316 (0.0368)	0.233 (0.0092)	0.241 (0.0123)	0.0901 (0.0026)	2.592 (0.174)	1.201 (0.0917)
	(24) Nanaimo River	0.243 (0.0033)	1.637 (0.0936)	1.306 (0.0556)	0.230 (0.0041)	0.235 (0.0102)	0.0852 (0.0079)	2.715 (0.196)	1.220 (0.0472)
	(25) Nitinat Lake	0.232 (0.0026)	1.719 (0.116)	1.348 (0.0347)	0.219 (0.0050)	0.231 (0.0096)	0.0817 (0.0091)	2.712 (0.395)	1.225 (0.0916)

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Appendix II. Continued

CLADE /SUBCLADE	(site#) LOCATION	FEMALE			MALE				
		GSW/ PL	GSW/ GSL	A1 24:22	rtP5exo1/ PL	rtP5exo2/ PL	lftP5Bp2W/ PL	rtP5exo1/ lftP5Bp2W	A1 24:22
Asia									
	(27) Ishikari River	0.218	1.510	1.288	0.220	0.231	0.0810	2.723	1.322
		(0.0046)	(0.0760)	(0.0658)	(0.0073)	(0.0036)	(0.0048)	(0.253)	(0.0488)
	(29) Lake Ohnuma	0.211	1.567	1.365	0.207	0.226	0.0771	2.687	1.2996
		(0.0135)	(0.129)	(0.0780)	(0.0018)	(0.0012)	(0.0014)	(0.0582)	(0.0651)
	(D) Black Sea	0.192	1.361	1.407	0.200	0.215	0.0826	2.433	1.243
		(0.0066)	(0.0723)	(0.0467)	(0.0014)	(0.0067)	(0.0067)	(0.192)	(0.0232)
Europe									
	(32) Baltic Sea	0.1388	1.0722	1.7354	0.1710	0.1822	0.0957	1.7906	1.6872
		(0.0009)	(0.1047)	(0.0482)	(0.0097)	(0.0082)	(0.0071)	(0.0970)	(0.0637)
	(36) IJsselmeer	0.1749	1.2754	1.5852	0.1965	0.1924	0.1075	1.8277	1.6898
		(0.0057)	(0.0898)	(0.0714)	(0.0031)	(0.0042)	(0.0015)	(0.0538)	(0.0719)
	(37) Gironde River	0.1530	1.1467	1.8452	0.1642	0.1666	0.0991	1.6588	1.7343
		(0.0052)	(0.0347)	(0.0874)	(0.0051)	(0.0090)	(0.0052)	(0.0358)	(0.0339)
	(38) Tamar River	0.1748	1.2505	1.8080	0.1807	0.1798	0.0942	1.9302	1.7071
		(0.0045)	(0.1113)	(0.0455)	(0.0006)	(0.0010)	(0.0091)	(0.1938)	(0.0351)
	(E) Hickling Broad (lake)	0.1731	1.2321	1.6408	0.2019	0.2009	0.1070	1.8871	1.6009
		(0.0068)	(0.0399)	(0.0355)	(0.0067)	(0.0078)	(0.0047)	(0.0228)	(0.0705)
E. americana									
	Duwamish River, WA	0.177	1.148	1.386	0.212	0.236	0.0571	3.716	1.399
		(0.0033)	(0.0799)	(0.0819)	(0.0032)	(0.0098)	(0.0016)	(0.105)	(0.018)

Appendix III. Pairwise genetic distances (Cavalli-Sforza & Edwards, 1967) using five allozyme loci. These values were used to determine correlation with mitochondrial DNA distances using Mantel's (1967) test. Numbers in parantheses represent locations of populations shown in Figure1

	(10)	(9)	(23)	(20)	(2)	(5)	(1)	(21)	(25)	(B)
(10) Tisbury										
(9) Edgartown Pond	0.1593									
(23) Grays H. Marsh	0.1275	0.1031								
(20) San Francisco	0.1217	0.0918	0.0198							
(2) St. Lawrence marsh	0.1585	0.1437	0.1119	0.1060						
(5) Waquoit Bay	0.0759	0.1906	0.2380	0.2421	0.3249					
(1) St. Lawrence R.	0.0576	0.1747	0.2256	0.2169	0.2603	0.0227				
(21) Columbia R.	0.3582	0.3694	0.3891	0.4254	0.3421	0.4247	0.3904			
(25) Nitinat Lake	0.2768	0.2793	0.2460	0.2813	0.1913	0.3949	0.3602	0.0712		
(B) Lake Texoma	0.1521	0.2030	0.1277	0.1219	0.0651	0.3236	0.2922	0.4167	0.2477	
(15) Mississippi R.	0.1279	0.1503	0.1032	0.0896	0.0288	0.2992	0.2500	0.3993	0.2394	0.0215