Hydrobiologia **359:** 125–147, 1997.
M. A. Simovich, C. Sassaman & D. Belk (eds), Studies on Large Branchiopod Biology and Conservation.

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Reproductive isolation and genetic differentiation in North American species of *Triops* (Crustacea: Branchiopoda: Notostraca)

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Key words: allozymes, androdioecy, *Triops longicaudatus*, *Triops newberryi*, unisexuality

Abstract

Electrophoretic analysis of 31 populations, ranging from California to Kansas, indicates that the North American tadpole shrimp *Triops longicaudatus* (LeConte) is actually a mixture of at least two reproductively isolated species. In the central United States, the predominant species is *T. longicaudatus*, which is found typically in ephemeral prairie pools. In the southwestern United States, the predominant species is *Triops newberryi* (Packard), which characteristically inhabits large playa pools. The two species coexist occasionally and in sympatric situations they are reproductively isolated from each other. The two species are genetically distinct at a level greater than is typical of conspecific populations. These genetic differences are correlated with subtle morphological differences and profound differences in reproductive biology. Both species represent complexes of bisexual and unisexual populations. Bisexual populations of *T. longicaudatus* are usually composed of males and females in approximately equal frequencies; bisexual populations of *T. newberryi* are composed of males in low frequencies and self-compatible hermaphrodites. Unisexual populations of both species apparently consist entirely of self-compatible hermaphrodites.

Introduction

The Notostraca is a small, widely distributed order of branchiopod crustaceans. Its two genera, *Triops* Schrank and *Lepidurus* Leach, are found in temporary and semipermanent aquatic habitats on all continents except Antarctica. Both genera have long and complex taxonomic histories; about 60 junior synonyms exist for the eleven or so species recognized by Longhurst (1955a). The history of notostracan taxonomy has seen the proliferation of a large number of names for various populations on each major continent followed, as a consequence of the monographs of Linder (1952) and Longhurst (1955a), by a major reduction to a few species, each with geographically widespread distribution. Neither Linder's nor Longhurst's views on the taxonomy of *Lepidurus* have survived intact (Lynch, 1966; 1970; Saunders, 1980a) but their complementary views on the classification of *Triops* have persisted, even attaining the status of dogma (*e.g.*, Tiwari, 1972) . Nevertheless, heterogeneity within some nominal species has been recently interpreted as indicating some intermediate degree of subspecific differentiation (Fryer, 1988; Brtek et al., 1984; Thiéry, 1987; Brtek & Thiéry, 1995). To date, however, there has been no compelling evidence of specific differentiation within any of the species of *Triops* as recognized by Linder (1952) or Longhurst (1955a).

Triops longicaudatus (LeConte) is a widespread inhabitant of temporary waters in arid areas throughout western North America, with populations also found in the Caribbean, South America, the Galapagos Islands, Hawaii, New Caledonia, Japan and Korea (Linder, 1952; 1960; Longhurst, 1955a; Akita, 1976; Yoon et al., 1992). The species is noteworthy in showing large amounts of morphological variation throughout its range, and in exhibiting substantial variation in sex ratio. The morphological variation among North

American populations alone prompted the description of five additional species (as *Apus*) over the last century (Packard, 1871; Rosenberg, 1947). Packard's three species (*T. lucasanus*, *T. newberryi*, and *T. aequalis*) were based on a very limited number of specimens from a small number of localities and Rosenberg's two species (*T. oryzaphagus* and *T. biggsi*) were based on specimens from unisexual populations collected from California rice fields. Linder (1952) surveyed a large number of museum lots, primarily from the US National Museum, and reanalyzed many of the characters used by Packard in describing North American forms (Packard, 1871; 1883). Linder documented a wide range of variability in many morphological characters and in sex ratio and viewed North American*Triops* as a polytypic species with many locally differentiated sexual populations as well as other, even more derived, parthenogenetic forms. Accordingly, he reduced Packard's and Rosenberg's species to junior synonyms of *T. longicaudatus*. He also extended the range of this species to include populations from the Galapagos Islands, the Hawaiian Islands, and Argentina. Longhurst (1955a) concurred with Linder (1952) on the synonymy of the North American populations and added to this synonymy all the species of *Triops* that had been described from the West Indies and South America. He also extended the range to include New Caledonia and Japan.

The existing morphological data strongly suggest substantial amounts of genetic variation within *Triops longicaudatus*. Bisexual populations vary considerably in characters such as the number of body rings, the number of terminal body rings which lack appendages, and patterns of spination of the telson. Furthermore, unisexual forms often differ from bisexual populations, and exhibit reduced variability of these traits. To date, there has been no analysis of genetic differentiation in North American *Triops* nor is there any information about the relatedness of bisexual and unisexual populations. We report here the results of studies on allozyme differentiation, population sex ratio, reproductive biology, and morphological variability in a series of populations distributed over much of western North America. Our results indicate that the polytypic *T. longicaudatus* defined by Linder (1952) and Longhurst (1955a) is actually a mosaic of several biologically different kinds of populations, representing at least two distinct species, that occasionally coexist without introgression.

Materials and general methods

Populations sampled

We have sampled 44 populations of *Triops* from throughout the western United States over the last sixteen years. The geographic distribution of sampling sites is shown in Figure 1 and collection details are given in Appendix 1. We collected morphological and genetic information from many of these populations; however, in some instances only distributional data were recorded. Most populations were sampled by collecting dry soil which contained desiccated cysts and subsequently rearing individuals in the laboratory. In those cases where ponds were full at the time they were visited, live *Triops* were sampled by dip net. Those individuals that were collected for genetic analysis were frozen on dry ice, and returned to the laboratory where they were stored at -70 °C. Other individuals were studied alive or were preserved for morphological analysis.

Laboratory Rearing

Samples of 100–200 ml of soil from field collections were hydrated in 38 l aquaria of demineralized water provided with aeration and fluorescent illumination at ambient temperatures (20–25 °C). Hatchlings were initially fed with pulverized fish food pellets. As the animals grew they were fed freshly killed, and later live, *Artemia*. Upon maturity, individuals intended for genetic analysis were scored for a variety of morphological characters and then frozen and stored at -70°C. Some individuals were reared from hatching to maturity entirely in isolation. From these we collected eggs that were air-dried and subsequently tested for viability.

Morphological characters

Individuals that were reared in the laboratory were typically scored for the following characteristics: (1) sex (determined primarily by the presence or absence of an egg sac on the eleventh thoracic appendage), (2) carapace length (measured from the anterior margin to the mid-dorsal terminus), (3) total body length (anterior margin of carapace to posterior margin of telson, not including the caudal furci), (4) total number of body rings, (5) number of terminal body rings lacking appendages (legless rings), and (6) the number of spines in the central row on the dorsal surface of the

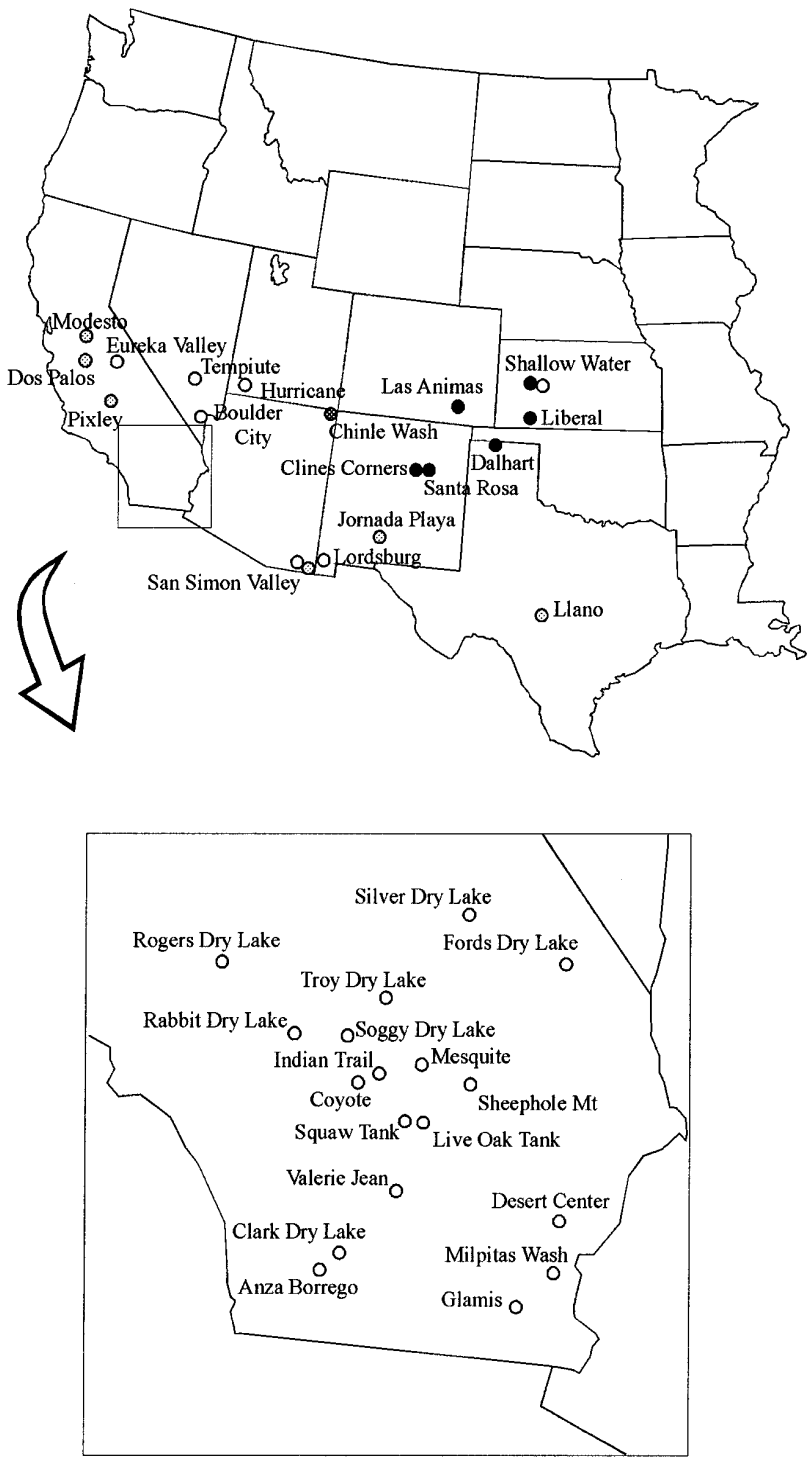

Figure 1. Geographic distribution of sampling sites of North American populations of *Triops*. Inset shows localities of southern California populations. Filled circles represent gonochoric populations of *T. longicaudatus*, dark stippled circles represent androdioecious populations of *T. longicaudatus*, light stippled circles represent unisexual populations of *T. longicaudatus*, unfilled circles represent *T. newberryi* (see text for details).

telson. The number of exposed body rings (those not covered in dorsal aspect by the carapace) was counted in some specimens. Exposed body rings and total body length were determined only on quiescent but unanesthetized living individuals.

Many of these characters have been dealt with in some detail by Linder (1952), and we followed his conventions in scoring characters. In summarizing the body ring data, incomplete rings were given a value of 0.5 ring, which was addded to the count of complete rings. For legless body rings, the origin of the most posterior appendage was scored to the nearest 0.5 ring. The anterior-most exposed ring was evaluated to the nearest 0.5 ring on living individuals with the carapace gently held down against the body.

Two meristic variables were derived from body ring counts: (1) covered body rings (total body rings minus exposed body rings) and (2) abdominal rings with legs (total body rings minus legless rings minus 11 thoracic rings). The ratio of carapace to body length was also calculated from measured variables. Total body length measurements and exposed ring counts were not made on either frozen specimens or preserved specimens since the normal tone of the body musculature was lacking. For these individuals, the two derived variables (covered body rings, and carapace ratio) could not be calculated.

Electrophoretic methods

Protein extracts were prepared by grinding individuals with a Teflon-tipped pestle in cold buffer (0.05 m Tris, pH 7.5) in 1 ml per 0.4 g wet weight. Extracts were then centrifuged in an IEC clinical centrifuge at approximately 190 RCF for ten minutes at 5 °C. Samples of the supernatant solution were frozen at −70 °C.

As needed, frozen extracts were allowed to defrost on ice and subjected to allozyme analysis. Allozymes were detected with electrophoresis using 12% starch gels prepared with one of three buffers and stained using the recipes of Selander et al. (1971) with occasional minor modifications. After an initial survey of a large number of systems, 17 enzymes encoded by 21 presumptive loci were found to be reliable and informative. For four very active enzymes, extracts were diluted 2:1. For three systems, agar overlays were used in the staining solution. Locus nomenclature, optimal buffer systems, and specifics of the staining procedure are given in Appendix 2. Alleles were scored according to decreasing anodal mobility (with 1 representing the most anodal form). After inital scoring of populations, individuals representing the extent of variation at a locus were reanalyzed to establish allelic identity.

Hierarchical branching patterns from UPGMA clustering of Nei's genetic distance and from restricted maximum likelihood using a brownian motion model of genetic change were obtained using the GENDIST, NEIGHBOR, and CONTML programs of PHYLIP 3.53 (Felsenstein 1989). CONTML was run at least 10 times with different input orders using the global rearrangement option. Nei's genetic distance is formulated for an infinite isoalleles model of mutation with all loci having the same mutation rate and assumes that change is by both genetic drift and mutation. The brownian motion model of the CONTML program assumes that change is by genetic drift only. The UPGMA clustering method assumes that gene substitution rates are the same in each lineage, while the maximum likelihood method allows for rates to vary (Nei, 1987; Felsenstein, 1985); the combined effects of migration, mutation and drift are likely to vary substitution rates between lineages. Trees were compared against the best tree using a pairwise z-test of the differences in likelihood on a locus-by-locus basis with the user-defined tree feature of the CONTML program.

Results

Preliminary observations

Heterogeneity among North American populations of *Triops* was indicated initially by morphological analyses of samples from the San Simon Valley of southeastern Arizona-southwestern New Mexico. Samples collected in 1981 from various ponds, stock tanks, and roadside ditches over an area about 13 by 32 km in the vicinity of Portal, AZ and Rodeo, NM were scored for several meristic characters, especially the total number of body rings and the number of legless body rings. The salient result was that two distinct morphotypes occurred in the area, differing in both of the segmentation variables. One form was characterized by approximately 36 body rings with the last six being legless; the other form was characterized by about 39 body rings with the last eight lacking appendages. The two forms also differed in reproductive characteristics. The shorter-bodied form consisted entirely of females whereas the longer-bodied was represented by some populations that were entirely female and other populations in which males were present, but generally infrequent (ca 15%).

The morphological heterogeneity in these Arizona populations was further substantiated by preliminary electrophoretic analysis using eight protein loci. Although there was virtually no genetic variation within samples within each morphotype, short-bodied individuals differed consistently from long-bodied forms at a substantial fraction of the loci analyzed. Among almost 400 individuals analyzed from ten ponds there were only six different 8-locus genotypes, but most short-bodied forms were of a single, 8-locus genotype and most long-bodied forms were of another.

Electrophoretic patterns

The genetic heterogeneity among morphotypes in the San Simon Valley of Arizona prompted a more extensive survey of *Triops* populations over a broader geographic scale and involving a larger suite of protein characters. A total of 399 individuals from 31 populations, ranging from California to Kansas, was analyzed for 21 loci (yielding a total of 63 alleles). The resulting allele frequencies are given in Appendix 3. (Populations are designated by letter codes that correspond to full site names and locality information in Appendix 1.)

The basic result of the preliminary observations in Arizona was repeated in the more extended survey: there are two distinct lineages of *Triops* populations that are distinguished from each other by substantial genetic differentiation. The complete separation of populations into two groups is supported by absolute genetic divergence at six loci (*Acph-3*, *Aat-2*, *Idh-1*, *Idh-2*, *Pept-C*, and *Acon-1*) and by substantial divergence of most populations at three others (*Aat-1*, *Gus*, and *Me*).

Clustering of populations by various methods and using different assumptions about the processes of genetic differentiation between populations yielded trees of consistent topology. Two such trees are illustrated in Figure 2, a UPGMA phenogram based on Nei's unbiased genetic distances (Figure 2A) and a maximum likelihood dendrogram based on sequential addition of populations to a growing tree (Figure 2B). The latter analysis represents the best topology of over 30,000 examined. The trees resulting from other analyses, such as Wagner trees, and resulting from other metrics of genetic distances, such as Cavalli-Sforza and Edward's chord measures, are not shown, but have similar topologies to those illustrated.

For cluster analysis, genetically identical populations were indexed to the location represented by the largest sample size. For example, 31 individuals from five ponds from southeastern Arizona (Sht, Tri, Ycm, Mll, and Mlh) were identical to each other at all loci; these populations are represented in Figure 2 by the upper branches labeled San Simon Valley (Sht). Similarly, two populations from eastern California, Eureka Valley (Eur) and Fords Dry Lake (Frd) are represented by one terminus (Eureka Valley), and five populations from California and New Mexico, Indian Trail (ITr), Valerie Jean (VaJ), Clark Dry Lake (Clk), Mesquite Dry Lake (Mes), and Lordsburg Playa (Ldb) are also combined (as Indian Trail). Also note that two localities, San Simon (Sht) and Shallow Water (ShW), are represented twice in each tree. Samples from these sites contained two discrete categories of genotypes that were absolutely differentiated from each other at a minimum of six loci (Appendix 3), implying the presence of multiple forms.

The Nei distance of 0.72 that separates the two principal clusters of populations in Figure 2A is three times larger than the distance at which each cluster is itself united (*ca*. 0.23) and is larger than the values measured between well-defined species in most other animal groups (Ayala, 1975; Nei, 1987). It is comparable to the values obtained for pairs of *Drosophila* species exhibiting substantial to complete levels of both pre- and post-zygotic isolation (Coyne & Orr, 1989). For purposes of clarity, we designate these two clusters of populations as *Triops longicaudatus* (the first 13 samples listed in Appendix 3 and the upper branches in each of the two trees of Figure 2) and *Triops newberryi* (the remaining samples and the lower branches). The explicit justification for this nomenclature will be developed below. Of the total of 63 alleles identified in our allozyme survey, 27 were found only in *T. longicaudatus* populations, 15 were unique to *T. newberryi*, and 21 were common to both species.

Sex ratio variation and modes of reproduction

Populations of *Triops longicaudatus* exhibit three distinct patterns of sex ratio. Some populations, primarily distributed in the eastern part of the range of samples, have sex ratios near equality with males being equally as frequent as females (Table 1). Other populations, from California, central Texas, southern Arizona, and northern Baja California, Mexico, are uniformly female. One population in northeastern Arizona (near Chinle Wash) has a strongly female-biased sex ratio. Populations of *Triops newberryi*, in contrast,

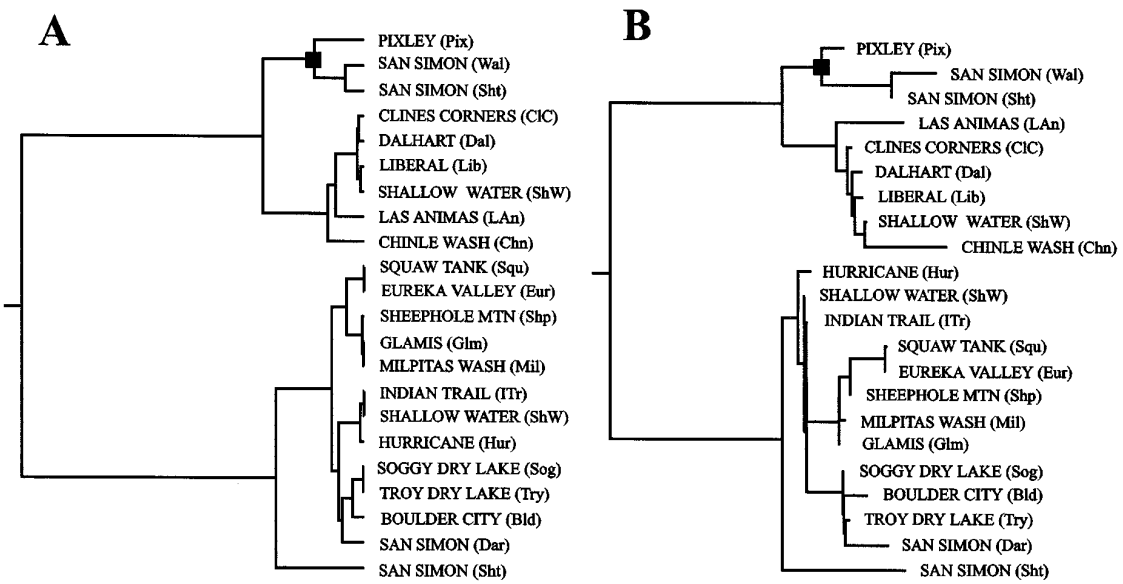

Figure 2. Genetic relatedness of *Triops* populations based on electrophoretic data. A. Phenogram generated by UPGMA clustering of Nei's unbiased distances. B. Dendogram generated by maximum likelihood neighbor joining of populations.

exhibit only female-biased or unisexual states (Table1), and males rarely exceed 16% of the population.

Previous laboratory studies on several populations of *Triops newberryi* have indicated that females from both female-biased and from unisexual populations, when reared entirely in isolation from hatching, nevertheless produce viable cysts (Sassaman, 1991). Furthermore, the pattern of appearance of males in clutches produced during unisexual reproduction in this species (Sassaman, 1991), by analogy with comparable patterns in the conchostracan *Eulimnadia texana* (Sassaman & Weeks, 1993), indicates that reproduction is by selfing hermaphroditism.

Females from eastern populations of *Triops longicaudatus* (those with sex ratios near equality) do not produce viable cysts when reared in isolation. The cysts that are produced are irregular in size, shape, and color; they lack the normal extra-embryonic coatings; and they do not hatch upon subsequent hydration. Limited experiments with females from the Chinle Wash population (the only female-biased population of *T. longicaudatus* that we have studied), however, have yielded some viable cysts produced by females reared in isolation. Individuals from unisexual populations of *T. longicaudatus* produce viable cysts when reared in isolation and histological evidence (Longhurst, 1955b; Akita, 1971; 1976) indicates that they are self-compatible hermaphrodites. (For a review of the reproductive biology of notostracans, see Sassaman (1991).)

We follow the terminology of Sassaman (1995: Figure 2) to denote the various populations of *Triops* in terms of the reproductive characteristics of their life cycles. Thus, bisexual populations (those containing males) include both those with an obligately outcrossing mode of reproduction (gonochoric) and those with a mixed mating system involving outcrossing and facultative selfing (androdioecious). Unisexual populations of North American *Triops* all presumably reproduce entirely by selfing.

Genetic correlates of reproductive biology

On the basis of allozyme differentiation (Figure 2) and reproductive characteristics (Table 1), there are five potentially distinct biological entities of North American *Triops*: unisexual and androdioecious populations of *T. newberryi*, and gonochoric, androdioecious and unisexual populations of *T. longicaudatus*. In *T. newberryi*, androdioecious and unisexual populations are not sharply demarcated from each other; there is a continuous gradation of sex ratios from 16% males to an apparent absence of males (Table 1). Because of this gradation, no population can be proven to lack males completely; we can only estimate from our data the maximum frequencies in which they might occur in

Table 1. Numbers of males and females collected or reared from various North American populations of *Triops.*

Population	Number of males	Number of females	Total	Proportion male
T. longicaudatus				
Shallow Water, KS	17	8	25	0.68
Liberal, KS	13	7	20	0.65
Dalhart, TX	30	25	55	0.55
Las Animas, CO	5	2	7	0.71
Santa Rosa, NM	3	0	3	1.00
Clines Corners, NM	72	62	134	0.54
Chinle Wash, AZ	9	48	57	0.16
Llano, TX	0	78	78	0.00
San Simon Valley, AZ (Sht)	0	321	321	0.00
San Simon Valley, AZ (Mlh)	0	44	44	0.00
San Simon Valley, AZ (YcM)	0	110	110	0.00
San Simon Valley, AZ (Wal)	0	509	509	0.00
Modesto, CA	0	23	23	0.00
Pixley, CA	0	2	2	0.00
Ejido Héroes de la Indepencia Baja (Norte)	0	4	4	0.00
T. newberryi				
Shallow Water, KS	2	18	20	0.10
San Simon Valley, AZ (Sht)	11	66	77	0.14
Hurricane, UT	3	84	87	0.03
Boulder City, NV	6	98	104	0.06
Eureka Valley, CA	1	88	89	0.01
Sheephole Mt., CA	28	145	173	0.16
Desert Center, CA	1	7	8	0.13
Indian Trail Dry Lake, CA	1	48	49	0.02
Coyote Dry Lake, CA	1	33	34	0.03
Troy Dry Lake, CA	1	18	19	0.05
Lordsburg Playa, NM	0	44	44	0.00
San Simon Valley, AZ (Dar)	0	281	281	0.00
Valerie Jean, CA	0	51	51	0.00
Glamis, CA	0	11	11	0.00
Milpitas Wash, CA	0	42	42	0.00
Soggy Dry Lake, CA	0	29	29	0.00
Squaw Tank, CA	0	70	70	0.00
Anza Borrego, CA	0	7	7	0.00

those populations from which they have not been identified. Furthermore, biological distinctions between androdioecious and all-female populations need not be sharp because there is a continuous gradation of population genetic effects depending upon the frequencies of males in the population (Sassaman, 1989; 1991; Otto et al., 1993). In the dendrograms of population relatedness (Figure 2), there is no distinct clustering of *T. newberryi* populations by reproductive type; populations with high male frequencies, as well as apparently unisexual populations, occur in each of the subbranches of both distance and maximum likelihood trees. Of the 36 alleles identified in our electrophoretic survey of *T. newberryi*, only one was unique to unisexual populations, whereas 9 were found only in sexual populations, and the remaining 26 were common to both types of populations.

In *T. longicaudatus*, in contrast, there is a clearer demarcation between populations differing in sex ratio, and reproductive differences are associated with the patterning of genetic relatedness. Unisexual populations, in particular, appear as a 'monophyletic' clade in both distance phenograms and maximum likelihood dendrograms (their common node is marked by a square in Figure 2), and the one androdioecious population (Chinle Wash) is the sister group to all of the gonochoric populations of *T. longicaudatus* in the distance phenogram (Figure 2A). Of the 48 alleles identified in *T. longicaudatus*, two were found only in asexual populations, one was unique to the androdioecious population, 17 were found only in sexual populations, and the remaining 28 were shared between at least two different kinds of populations.

Conventional statistics of genetic variability were calculated from the gene frequency data for those populations in which at least five individuals were scored for the 21 loci. The number of alleles per locus and the number of loci polymorphic (at the criterion that the most common allele was in a frequency less than 0.99) were obtained by direct count; the effective number of alleles at each locus, n_e, was calculated from the frequencies of each allele, p_i, as $n_e = (\Sigma p_i^2)^{-1}$, and expected heterozygosity at each locus was calculated from the binomial expansion of the individual allele frequencies. These statistics were then averaged over loci and over populations for each of the reproductive types (Table 2).

In *T. longicaudatus*, there is a strong correlation between reproductive mode and genetic diversity. Obligately sexual (gonochoric) populations are quite variable, by any measure, and the variability is comparable to that found in other species of invertebrate animals (*e.g.*, Powell, 1975; Nevo, 1978; Nei, 1987). Unisexual populations, in contrast, are completely lacking in detectable variation. Although these populations sometimes differ from each other at some loci (Appendix 3), there is no intra-populational variation. The one androdioecious population from Chinle Wash is intermediate in all measures of genetic diversity.

Genetic variance is lower, on average, in *T. newberryi* that in *T. longicaudatus*. Androdioecious populations have somewhat more variance than unisexual ones, but unisexual populations are not completely homogeneous, as they are in *T. longicaudatus* (Table 2). Comparisons between the two species, of populations with the same reproductive mode, suggest that a primary determinant of genetic variation is reproductive mode.

Geographic patterns of genetic differentiation

Although distance phenograms, such as the one illustrated in Figure 2, describe the genetic relatedness of populations, and are based on the same data that provide information on genetic exchange between populations, they do not include any explicit information on the geographic proximity of populations. To examine the spatial scale of population differentiation we used methods recently developed by Slatkin (1991, 1993). His method investigates the relationship between geographic distance and genetic difference by relating isolation by distance to average coalescent times of genes. Under this model, pairwise estimates of migration between populations are inversely correlated with the number of steps between them (Slatkin, 1993). $\hat{M}$, the value of Nm (the product of population size and per capita migration rate) that would be estimated for a pair of populations from their pairwise genetic correlation (θ) in a one-dimensional stepping-stone model, is approximately equal to Nm/i, where i is the number of steps separating the two populations. In a one-dimensional array of populations, the expected slope of the regression of $\log_{10}(\hat{M})$ versus $\log_{10}$(distance) is -1.0 and in a two-dimensional array, the expected slope is -0.5. Simulations indicate that values of $\hat{M}$ based on fewer than ten highly variable, polymorphic loci can show a pattern consistent with isolation by distance despite considerable variation (Hellberg, 1994).

Pairwise values of $\hat{M}$ were obtained from estimates of θ between pairs of populations using the computer program of Slatkin (1993). The $\log_{10}$ of the estimated $\hat{M}$ values were plotted against the $\log_{10}$ of the geographic distances for pairs of populations within *T. newberryi* and within sexual populations of *T. longicaudatus*. The analysis for *T. newberryi* was based on 9 populations and 7 loci and the analysis for *T. longicaudatus* on 5 populations and 14 loci. Populations with fewer than 5 individuals sampled were excluded because variability in sample sizes can lead to negative $\hat{M}$ values. Since the number of steps separating actual populations is measured with error, both ordinary-least-squares (OLS) and reduced-major-axis (RMA) regression were used to characterize the relationship of gene flow and distance (Hellberg, 1994).

Sexual populations of *T. longicaudatus* exhibit a strong relationship between genetic distance and geo-

Table 2. Comparisons of genetic diversity among North American populations of *Triops* by species and reproductive mode. Mean values and standard deviations among populations are shown.

Species Reproductive system	Number of populations	Number of alleles	Effective number of alleles	% Loci polymorphic	Expected individual heterozygosity
T. longicaudatus					
Unisexual	5	1.00 (0.00)	1.00 (0.00)	0.00 (0.00)	0.000 (0.000)
Androdioecious	1	1.14	1.09	14.3	0.054
Gonochoric	4	1.65 (0.05)	1.28 (0.04)	47.6 (6.73)	0.144 (0.015)
T. newberryi					
Unisexual	6	1.03 (0.02)	1.01 (0.01)	3.1 (2.4)	0.006 (0.006)
Androdioecious	5	1.13 (0.09)	1.04 (0.04)	12.4 (8.0)	0.027 (0.026)

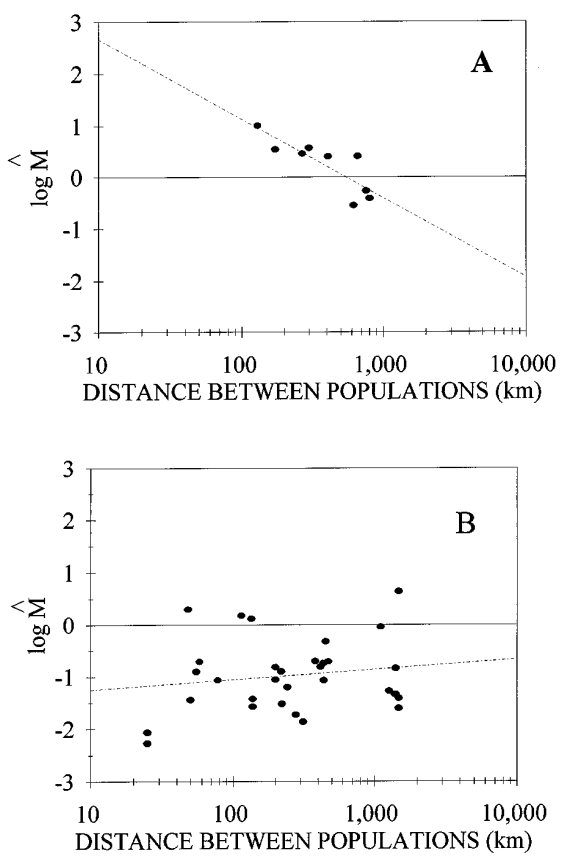

Figure 3. Geographic patterns of genetic differentiation in *Triops*. Regression of estimated migration rate between pairs of populations on the geographic distance between them. A. *Triops longicaudatus*. B. *Triops newberryi*.

graphic distance (OLS: $f(x) = -1.51\,x + 4.15$; $r^2 = 0.70$) (Figure 3A). The form of the relationship implies that the effective genetic exchange between populations is inversely related to the geographic distance between them. As the genetic exchange between populations approaches the value $Nm = 1$ (estimated here by $\log_{10} \hat{M} = 0$) populations are no longer genetically connected by sufficient migration to overcome differentiation by local forces such as drift and weak differentiating selection, and such populations become relatively uncoupled from each other as evolutionary units (*c.f.*, Wright, 1931). In *T. longicaudatus*, the separation distance at which this value is attained is approximately 500 km.

There is no relationship between migration and distance in *T. newberryi* (OLS: $f(x) = 0.203x - 1.48$; $r^2 = 0.03$) (Figure 3B). The slope of the regression actually suggests that genetic difference decreases with geographic distance, and estimated migration rates between almost all pairs of populations (over about 2 orders of magnitude of geographical scale) are below the value typically associated with genetic connectedness.

The range of *T. longicaudatus* almost completely overlaps that of the obligately sexual anostracan *Branchinecta packardi* and the two are often sympatric. Over a larger area, but sharing 4 of the 5 pools with *T. longicaudatus*, 9 populations of *B. packardi* also have a regression slope less than -1.0 (Fugate, unpubl.) suggesting that these two passively-dispersed species share migration patterns. Data for other androdiecious species with ranges overlapping that of *T. newberryi* are unavailable. However, *T. newberryi* populations have a relationship between $\hat{M}$ and geographic distance similar to those of cyclic parthenogenetic cladocerans (Innes, 1991; DeMelo & Hebert, 1994). Both groups have mating systems that feature selfing interrupted by periods of sexual reproduction. Without more data, the influence of the mating system on the relationship cannot be isolated from the influence of founder effects

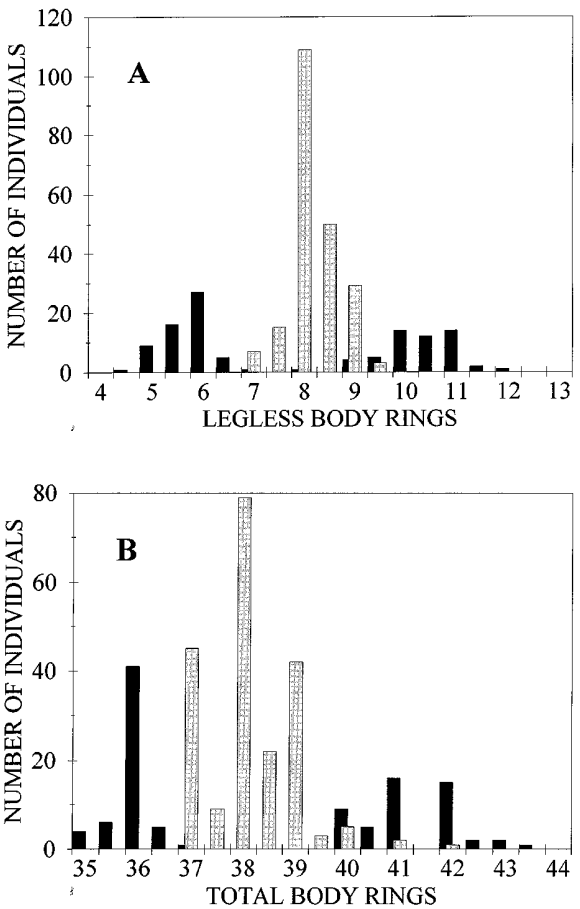

Figure 4. Frequency distributions of meristic character values in *Triops longicaudatus* (solid bars) and *T. newberryi* (stippled bars). A. Legless body rings. B. Total body rings.

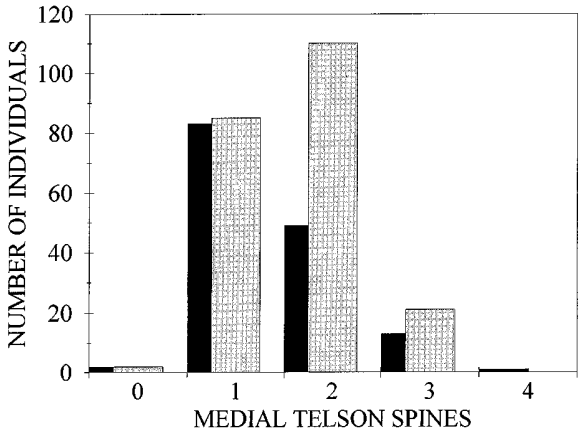

Figure 5. Frequency histograms of telson spine counts in *Triops longicaudatus* (solid bars) and *T. newberryi* (stippled bars).

or fluctuating selection. The latter two have been proposed as alternate hypotheses to explain large genetic differences between nearby pools in some freshwater crustaceans (e.g., Hebert, 1974; Lynch, 1987; Hairston & Dillon, 1989; Boileau et al., 1992).

Morphological differentiation between species and among populations

The genetic differentiation between the two North American *Triops* species (Figure 2) is accompanied by significant, but subtle, morphological differentiation in the numbers of legless body rings and the total body ring counts (Figure 4). The two species clearly differ in these two characters, but the nature of the difference is complex. For both variables, the distribution of character states for *T. newberryi* lies between the two modes of a bimodal distribution representing the character states for *T. longicaudatus*. The two species also differ in the number of spines in the central row on the dorsum of the telson (Figure 5). Rank-order comparsions of these two distributions indicate a highly significant difference, despite extensive overlap in values. Analysis of carapace ratio did not provide any clear pattern of differentiation between the two species.

These four morphological characters were also used to examine patterns of morphological differentation between populations within each species. Mean values and standard deviations for the four characters are given in Tables 3 and 4 for populations of *T. newberryi* and *T. longicaudatus*, respectively. Data are summarized only for populations in which at least five individuals were measured. These data were examined by ANOVA to investigate intraspecific heterogeneity. Parametric analyses were followed by pairwise multiple comparisons using the Student-Newman-Keuls test, and non-parametric (Kruskal-Wallis rank order) tests were followed by multiple comparisons using Dunn's test. Since population variances were heterogeneous in all comparisons (except for telson spination in*T. longicaudatus* males), and the general pattern of differences was comparable between parametric and non-parametric analyses, only the latter are presented here.

In *T. newberryi*, sample sizes were too small to allow statistical comparisons among males from different populations. Comparisons among females, however, generally indicated that sexual populations do not differ substantially from each other, nor do sexual populations consistently differ from unisexual popu-

Table 3. Morphological variation in female *Triops newberryi*.

Population	Legless rings			Total rings			Carapace ratio			Telson spines		
	N	Mean	S.D	*N*	Mean	S.D.	*N*	Mean	S.D.	*N*	Mean	S.D.
Kansas												
Shallow Water	17	8.44	0.50	13	38.8	0.44	12	0.445	0.035	13	2.00	0.41
New Mexico												
Lordsburg Playa	6	8.25	0.99	6	39.2	0.98	6	0.470	0.023	6	1.33	0.52
Utah												
Hurricane	37	8.39	0.50	37	38.7	0.64	37	0.452	0.034	37	1.68	0.63
Nevada												
Boulder City	23	7.96	0.37	23	38.1	0.52	23	0.458	0.015	23	1.61	0.66
California												
Coyote Dry Lake	33	8.36	0.46	33	37.4	0.45	33	0.480	0.029	33	1.79	0.60
Fords Dry Lake	5	7.40	0.22	5	37.2	0.45	5	0.456	0.022	5	1.80	0.45
Glamis	6	7.83	0.26	6	38.3	0.52	6	0.458	0.021	6	2.33	0.52
Indian Trail Dry Lk.	6	8.33	0.52	6	38.2	0.26	6	0.415	0.026	6	2.17	0.75
Sheephole Mt.	14	8.14	0.46	14	38.2	0.46	14	0.446	0.060	13	1.31	0.63
Soggy Dry Lake	17	8.18	0.30	17	38.1	0.28	17	0.459	0.035	17	1.53	0.72
Squaw Tank	12	8.25	0.40	12	38.5	0.48	12	0.460	0.040	11	2.00	0.63
Valerie Jean	20	8.10	0.21	20	37.1	0.15	19	0.440	0.014	20	1.50	0.41

Table 4. Morphological variation in male and female *Triops longicaudatus.*

Population	Legless rings			Total rings			Carapace ratio			Telson spines		
	N	Mean	S.D.	*N*	Mean	S.D.	*N*	Mean	S.D.	*N*	Mean	S.D.
Kansas												
Liberal ♀	3	10.33	0.76	3	40.5	0.50	3	0.433	0.032	3	1.00	0.00
♂	8	13.4	0.64	8	41.8	0.59	8	0.384	0.017	8	1.13	0.35
Shallow Water ♀	7	9.64	0.95	6	41.5	0.84	5	0.466	0.045	3	1.33	0.58
♂	13	12.6	1.00	9	42.1	0.73	8	0.406	0.037	13	1.14	0.38
Texas												
Dalhart ♀	9	10.00	0.79	8	41.1	0.79	8	0.379	0.038	8	1.00	0.53
♂	13	12.3	0.66	9	42.3	0.80	9	0.377	0.050	9	1.33	0.71
New Mexico												
Clines Corners ♀	22	10.45	0.64	22	41.7	0.84	18	0.393	0.027	22	1.23	0.43
♂	20	12.9	0.49	20	42.7	0.59	20	0.353	0.017	20	1.05	0.39
Arizona												
Chinle Wash ♀	12	10.67	0.58	11	40.6	0.80	11	0.445	0.034	8	2.50	0.93
San Simon Valley (Mlh) ♀	6	5.17	0.26	6	35.8	0.26	6	0.418	0.032	6	1.50	0.55
San Simon Valley (Mll) ♀	9	5.61	0.42	9	36.2	0.25	9	0.426	0.032	9	1.67	0.50
San Simon Valley (Sht) ♀	7	5.43	0.35	6	36.1	0.67	6	0.473	0.014	7	2.00	0.58
San Simon Valley (Tri) ♀	5	5.50	0.61	5	35.6	0.42	5	0.420	0.032	5	1.80	0.45
San Simon Valley (Wal) ♀	20	6.13	0.36	20	36.0	0.28	20	0.471	0.020	20	2.10	0.79
San Simon Valley (Ycm) ♀	8	5.88	0.23	8	35.8	0.37	8	0.433	0.020	8	1.50	0.53

lations. For legless body rings, only Fords Dry Lake differed from other populations (Shallow Water and Hurricane); no other pairwise comparsions were significant. Similarly, for carapace ratio, only the Coyote Dry Lake site was different (from Indian Trail and Valerie Jean). There were no significant differences between any populations for telson spination. The variation in total body rings was more complex. Individuals from Valerie Jean differed from most other populations, and Fords Dry Lake and Coyote Dry Lake differed from Lordsburg, Shallow Water and Hurricane. In general, populations that tend to be most different are those that lack males, but there is no clustering of populations into androdiecious and unisexual groups.

In *T. longicaudatus*, sample sizes were sufficient to allow comparisons among populations for both males and females. Since males are not present in all populations, these comparisons were conducted independently. In females, variation in carapace ratio is generally limited. Two populations, Santa Rosa and Dalhart, have relatively short carapaces and these are significantly different from some, but not all, of the other populations. For telson spination, non-parametric tests indicated no significant pairwise differences. The ring characters, however, showed strong correlations with reproductive type; all of the significant pairwise differences were between bisexual and unisexual populations.

In male *T. longicaudatus*, individual populations differed from each other, but with no consistent pattern across characters. Specifically, no population pairs differed for telson spination, Liberal differed only from Dalhart for legless rings, and only from Santa Rosa for total rings, whereas both Liberal and White Woman differed from Santa Rosa on carapace ratio, but not from each other.

With respect to the morphological characters we examined, females of *T. newberryi* are relatively homogeneous, irrespective of their geographical origin and whether or not they are from sexual populations. *Triops longicaudatus* females are more variable, but the principal variation arises from meristic differentiation between bisexual and unisexual populations. The population-level analysis supports the pattern obtained when data are aggregated over populations. The two modes in the frequency distributions of meristic characters in *T. longicaudatus* (Figure 4) are created by the discontinuity in character states of bisexual and unisexual populations. Males, although infrequent in *T. newberryi*, and not particularly variable in *T. longicaudatus*, nevertheless differ from females. The morphological data thus defines five entities, unisexual and bisexual females of *T. longicaudatus*, females of *T. newberryi*, and males of each species. We have pooled all of our morphological data into these categories, including information on additional character states not routinely measured on all individuals (see Methods). Statistics for the seven characters are summarized in Table 5.

Several aspects of these additional characters are worth noting. First, the number of abdominal body rings with legs is relatively constant among all the groups of North American *Triops*, varying only from 18.3 to 19.9 (Table 5). Since the number of thoracic rings is fixed at 11 (we found no exceptions), the variation in total body rings is highly correlated with the number of legless abdominal rings. Second, the number of rings covered by the carapace is relatively constant, varying from 10.3 to 13.2 (Table 5). In general, the carapace extends dorsally to cover the 11 thoracic rings. However, in unisexual *T. longicaudatus*, and to a lesser extent in *T. newberryi* females, the carapace extends farther back. Perhaps this posterior extension of the carapace, which might impede successful sperm transfer during mating, is related to the change from outcrossing to selfing in these forms. Third, the meristic character showing the largest variation among forms is the number of rings extending beyond the carapace (Table 5). Since it varies far more than the number of covered rings, this character is correlated (inversely) with the carapace ratio.

Both species are sexually dimorphic for most of the characters we examined. Males typically have more body rings, more legless abdominal rings, fewer body rings covered by the carapace, and a carapace that is shorter in relation to body length (Table 5). In gonochoric populations of *T. longicaudatus*, there is a marked sexual dimorphism associated with the armature at the base of the caudal furci. In males, the spines are much broader, and often flattened into scale-like structures, and the proximal part of the furca is often noticeably swollen. Gurney (1924) first described this dimorphism in *T. granarius*. In the androdioecious populations of both species this dimorphism is lacking; the shape of the furca and the nature of its spination does not differ perceptibly between males and females.

Geographical patterns of distribution

The geographical distribution of the two species, with populations of *T. longicaudatus* further differentiated by reproductive mode, is illustrated in Figure 1. All

Table 5. Summary of morphological characters of *Triops longicaudatus* from bisexual and unisexual populations and *Triops newberryi*.

Character	*Triops longicaudatus*									*Triops newberryi*					
	Males			Females			Unisexuals			Males			Females		
	Mean	*N*	SD	Mean	*N*	SD	Mean	*N*	SD	Mean	*N*	SD	Mean	*N*	SD
Total Body Rings	42.33	49	0.704	41.26	50	0.910	35.94	58	0.364	39.45	11	0.723	38.13	208	0.856
Abdominal Body Rings (with legs)	18.44	48	0.836	19.92	50	0.950	19.19	57	0.581	18.32	11	0.681	18.92	208	0.817
Legless Body Rings	12.76	57	0.774	10.31	53	0.755	5.75	60	0.483	10.04	12	0.582	8.21	213	0.485
Covered Body Rings	10.27	26	0.827	11.07	33	1.25	13.18	22	0.664	10.25	4	1.50	12.32	71	1.14
Exposed Body Rings	32.15	27	1.39	30.04	33	1.37	22.83	23	0.763	29.62	4	1.70	26.12	71	1.58
Carapace Ratio	0.375	48	0.037	0.414	45	0.044	0.449	58	0.033	0.417	11	0.042	0.458	212	0.035
Telson Spines	1.15	46	0.470	1.41	42	0.757	1.87	60	0.650	1.64	11	0.809	1.69	207	0.647

of these records are from localities examined by us as indicated in Appendix 1. Populations of *T. newberryi* are not differentiated by reproductive type because, for many localitites, sample sizes are too small to unambiguously assign populations as being either bisexual or unisexual. The insert shows the localities of southern California populations; all of which are *T. newberryi.* Not shown are two locations from Baja California. We have reared unisexual *T. longicaudatus* from a site in northern Baja California (Ejido Héroes de la Indepencia) and female *T. newberryi* from central Baja California (near Bahía de los Angeles).

Some literature records can be identified from illustrations and/or morphological descriptions. For example, specimens from Wyoming (Linder, 1952), Montana (Mail, 1934), and eastern Colorado (Saunders, 1980b) are identifiable as *Triops longicaudatus* on the basis of ring counts and male morphology, and this species was reported (as *T. lucasanus*) from the southern end of Baja California (Packard, 1871). Literature records are not sufficiently precise to identify additional populations of *T. newberryi*.

The two species differ somewhat in their geographic distributions (Figure 1). *Triops newberryi* is found primarily in the southwestern states, but may extend farther north into the Great Basin and farther south into central Mexico. Maeda-Martinez (1991), for example, reports both sexual and asexual populationsof*Triops* throughout Mexico, but does not map the two species explicitly. Although generally distributed west of the continental divide, at least some isolated populations occur in the central US (*e.g.*, in western Kansas). We have not detected any clear geographical pattern to the distribution of bisexual versus unisexual populations of *T. newberryi*. *Triops longicaudatus* is more geographically widespread, and populations of different reproductive modes appear to be highly patterned in space. Gonochoric populations are found primarily to the east of the continental divide, from northern New Mexico and Texas into eastern Colorado and western Kansas. Additional populations of this form probably extend into Wyoming and Montana. Unisexual populations occur to the south and west of the gonochoric populations, in a band ranging from central Texas through southern New Mexico and Arizona into the Central Valley of California. These populations also may extend into central Mexico. The only known androdioecious population of *T. longicaudatus*, in northwestern Arizona, lies between the gonochoric and unisexual populations. The geographic range of the androdioecious form is otherwise unknown; it may be narrowly endemic.

Our sampling of habitats was conducted primarily during their dry phase; therefore, we have limited information on ecological conditions during the ponding phase. However, ponds were sampled in the San Simon Valley early in this study; physical and chemical features have been described for a number of our California localities (Kubly, 1982); and some inferences may be made from our on-site observations during the dry phase. Generally, *T. longicaudatus* inhabits small pools; *T. newberryi* is more characteristic of larger alkaline playas. The unisexual form of *T. longicaudatus* is often associated with transient or disturbed habitats, such as roadside ditches, recently created stock-watering tanks, and agricultural fields.

The two species coexist within individual ponds in the San Simon Valley; field sampling during the summer of 1981 confirmed that androdioecious *T. newberryi* and unisexual *T. longicaudatus* are not only syntopic, but also have synchronic life cycles in southeastern Arizona. In laboratory culture, the two species

also have similar phenologies and life histories (Weeks, 1990; Weeks & Sassaman, 1990). Although both species inhabit some ponds in the valley, most of the habitats we examined (roadside ditches and artificial impoundments) were occupied only by unisexual populations of *T. longicaudatus*. The two species also coexist at our Shallow Water, Kansas, site where sexual populations of both species occur without any genetic indication of introgression (Appendix 3).

Discussion

The North American species of Triops

Although this contribution is not intended to be a formal taxonomic revision of either North American *Triops*, or of those populations elsewhere that are currently subsumed under *T. longicaudatus*, some comment on nomenclature is in order. Our evidence of multiple species of *Triops* creates a need to establish the nomenclature of the forms. The oldest recognized description of *Triops* from North America is that of *Triops longicaudatus* (Le Conte, 1846); the prior description of *T. obtusa* (James) is generally not recognized since the original description was fragmentary and unaccompanied by any type material. Packard (1871) briefly described three additional species: *T. lucasanus*, *T. newberryi* and *T. aequalis*. He amplified those descriptions and provided figures in his monograph on North American branchiopods (Packard, 1883). Two additional species, *T. oryzaphagus* and *T. biggsi*, were subsequently described from California rice fields by Rosenberg (1947). All of Packard's (1871) and Rosenberg's (1947) species were re-evaluated by Linder (1952), who examined available type material of all forms. The principal characteristics of these various species, as based on the original description or Linder's subsequent re-description of type materials, is summarized in Table 6.

In compiling the table, we have standardized ring counts, carapace ratios, and telson spine counts to the conventions used in our measurements. For example, some authors regard the terminal segment as a body ring; we follow Linder (1952) in regarding it as a telson. Various conventions have been used to determine carapace length, we have used the midline length. Telson spine counts vary in the literature depending upon whether only the medians are reported (as we do) or whether the posterior marginal spines are also included (see Longhurst, 1955a: Figure 5). LeConte's (1846) description of *T. longicaudatus* is somewhat limited, and little of quantitative value can be extracted from it other than that his specimens had about 15 legless body rings. He actually misinterpreted this region as being a post-anal tail, hence the specific epithet. The only other quantitative information on supposedly *bona fide T. longicaudatus* is in Packard's (1883) monograph. The value of this monograph is compromised by the large number of errors it contains (Lynch, 1964), but we know of no other explicit description of *T. longicaudatus*. Packard's (1883) redescription of males and females was based on LeConte's type and additional specimens from Texas. Quantitative data on Packard's (1871) and Rosenberg's (1947) species (Table 6) are based on the original descriptions and Linder's (1952) re-descriptions.

Our analysis of morphological variation within single populations of *Triops*, and between populations that share substantial genetic identity, indicate that slight meristic differences are not substantive indicators of genetic differentiation. Thus, we see no basis for distinguishing between *T. longicaudatus* (LeConte, 1846) and *T. lucasanus* (Packard, 1871). Morphological characters of both males and females from gonochoric populations in the central United States correspond very closely to characters of the type specimens.

The nomenclature of our second species is more problematic. There are two available names: *T. newberryi* (Packard, 1871) and *T. aequalis* (Packard, 1871), neither of which were described from many specimens or localitites. Packard (1883) regarded *T. newberryi* to be related closely to *T. longicaudatus*, and quite distinct in morphology from *T. aequalis*. Quantitative characters (Table 6), however, suggest that *T. newberryi* is as similar to *T. aequalis* in some characteristics (*e.g.*, carapace ratio and telson spination) as it is to *T. longicaudatus* in others. Our second species corresponds to one of these two forms, but we cannot unambiguously eliminate either. Since the reported geographical distribution of *T. newberryi* is more western, and since *T. newberryi* has page priority over *T. aequalis* (Packard, 1871) we tentatively restore *T. newberryi* as a valid species with *T. aequalis* as its junior synonym.

Linder (1952) viewed the body ring data for North American *Triops* as representing a 'continuous series', ranging from the shortest-bodied forms (*T. oryzaphagus*) to the longest-bodied forms (*T. longicaudatus*) and, largely on that basis, he synonymized all the described forms. Indeed, our morphological data, aggregated over all samples (Figure 4B) would be completely consistent with his interpretation. How-

Table 6. Comparison of morphological characters on type materials of North American *Triops*.

		Total body rings	Legless body rings	Exposed rings	Carapace ratio	Telson spines	Source[1]
T. longicaudatus	♂	?	13–15	31	.39	1	1, 4
	♀	?	9	27			
T. lucasanus	♀	43 (38–40)	12	32	.39	1	2, 3
	♂	39	10	28	.39	1	
T. newberryi	♀	40	10	28	.43	2–3	2, 3
T. aequalis	♂	36–38	10	22	.48	2	2, 3
	♀	38.5	8	24	.52		
T. oryzaphagus	♀	34.5–35	5	20.5	.53	1–2	2, 5
T. biggsi	♀	35.5–36	6	19.8	.62	1–3	2, 5

[1] Sources: 1: LeConte (1846); 2: Linder (1952); 3: Packard (1871); 4: Packard (1883); 5: Rosenberg (1947)

ever, removal of the counts for *T. newberryi* creates bimodal, rather than continuous, frequency distributions for the morphological traits in the remaining populations. Indeed, for almost all the characters we examined, samples of *T. newberryi* are intermediate between the bisexual and unisexual populations of *T. longicaudatus* (Table 5). What then is the appropiate nomenclature for the short-form shrimps? The answer to this question depends, in part, on the species concept that one chooses to apply. The form is distinct in morphology, and its reproductive biology seems to indicate that these populations are now on an evolutionary trajectory that is independent of that of sexual populations of *Triops longicaudatus.* On that basis, the short-form could be recognized as a separate taxonomic entity. Our material clearly encompasses the morphological variation in *Triops oryzaphagu*s and *T. biggsi,* which themselves are insufficiently distinct to be regarded as separate species. Thus, *Triops oryzaphagus* (which has page priority over *T. biggsi*) would be an appropriate name. Alternatively, this form may be viewed as simply a derivative group of populations that has recently arisen from *T. longicaudatus*, but that is nevertheless substantially similar in basic genetical characteristics. Until there is a further genetic analysis of the entire spectrum of unisexual populations, we prefer the conservatism of the latter view. Thus, we retain these populations in *T. longicaudatus*.

The status of other supposed synonyms of Triops longicaudatus

The final resolution of the status of unisexual populations of *T. longicaudatus* will certainly depend upon extensive analysis of material from non-North American localities. All specimens collected elsewhere, yet supposedly synonymous with *T. longicaudatus*, have been female. Two South American forms, *Triops pampeanus* (Ringuelet, 1944) and *Apus frenzeli* (Thiele, 1907) from Argentina, as well as two forms from the West Indies, *Apus guildingii* (Thompson, 1834) and *Apus domingensis* (Baird, 1852), were added to the synonymy of *T. longicaudatus* by Linder (1952, 1960) and Longhurst (1955a). Morphometric analyses of museum collections from Argentina have indicated the presence of two distinct morphotypes based on the patterns of spination on the telson and the surface structures of the resting eggs (César et al., 1991; 1993). These forms were nevertheless viewed as populations of *T. longicaudatus*, possibly varying from each other at the subspecific level. West Indian material (based on new collections) was examined by Linder (1960) who had specimens from Aruba, Bonaire, and Curacao. He viewed these specimens as falling within the continuum of character values for North American populations, indicating synonymy with *T. longicaudatus*.

Populations distributed elsewhere than the Americas were first reported either by Linder (1952) or Longhurst (1955a), or were discovered subsequent to their monographs. Thus, these populations – in the Galapagos Islands (Linder, 1952, 1960; Brendonck et al., 1990), Hawaii (Linder, 1952, 1960), New Caledonia (Longhurst, 1955a), Japan (Longhurst, 1955a; Akita, 1972, 1976) and Korea (Yoon et al., 1992) – have not had complex taxonomic histories. Isaac's (1970) record of *T. longicaudatus granarius* (for a form intermediate between *T. longicaudatus* and *T. granarius*)

from southern India is the exception, but this record is questionable.

Our finding that profound genetic differences between *Triops* species are accompanied only by subtle morphological differences raises serious doubts about the extensive synonymy of *T. longicaudatus*. Although some populations, particularly those associated with agricultural practices, may have been transported long distances by human commerce relatively recently, it seems unlikely that most non-North American populations have had extensive gene flow with North American populations in the recent past. Our estimates of isolation distances suggest that, even under the best of circumstances, populations lose genetic cohesion when separated by more than a few hundred km. Populations in the West Indies, in South America, and throughout the Pacific, are separated from North American populations, and from each other, by far larger distributional gaps. Furthermore, we have been unable to identify non-North American material with either of our unisexual groups. A preliminary morphological comparison of several specimens from Salta Province in northern Argentina, and a large lot of material from Aruba with unisexual *T. longicaudatus* and *T. newberryi* was inconclusive. Meristic counts did not clearly correspond to either of the two North American species. We therefore believe that the status of all non-North American forms attributed to *T. longicaudatus* requires re-evaluation.

Generalizations to other Notostraca

A corollary of our analysis of *T. longicaudatus* is that the morphological and life history variability of other nominal species of Notostraca may also reflect genetic differentiation and speciation. Most other tadpole shrimps have extensive synonymies; many have extensive ranges with large discontinuities in their distributions; many are morphologically variable; and some show significant variation in reproductive mode. Variation in *Lepidurus* Leach has received some attention, and the synonymies of Linder and Longhurst have not been substantiated by the more recent examination of North American and European forms. There has been far less attention to questions of species identity within *Triops*. *Triops cancriformis* (Bosc), for example, has an extensive synonymy (Longhurst, 1955a), exhibits geographic variation in sex ratio (and presumably reproductive mode) that is comparable to that seen in North America (Zaffagnini & Trentini, 1980), and is morphologically heterogeneous on both local (Hempel-Zawitkowska, 1968) and larger scales (Fryer, 1988). Some authors recognize subspecies of *T. cancriformis* (*e.g.*, Thiéry, 1987; Brtek & Thiéry, 1995), but stronger isolation and genetic differentiation may exist. *Triops granarius* (Lucas) also exhibits substantial morphological heterogeneity over its extensive geographical range (Barnard, 1920; 1929; Gurney, 1924; 1925; Thiéry, 1987; Tiwari, 1951; 1954; Karande & Inamdar, 1959; Rayner & Bowland, 1985; Shanbhag & Inamdar, 1968; Meintjes et al., 1994; Hamer & Rayner, 1995). Although unisexual populations of *T. granarius* have not been reported (nor has evidence of hermaphroditism) there are female-biased populations (Sassaman, 1991) and populations in which the normal sexual dimorphism of the caudal furci is lacking (Gurney, 1924; 1925), as we have found it to be in *T. newberryi*. The lesser variation in *T. australiensis* (Spencer & Hall) is accompanied by a simpler synonymy; nevertheless, the variation that is present (Main, 1953) may have a genetic component worthy of examination.

Concluding remarks

Triops has a long fossil record; specimens assigned to this genus are reported from the Permian of Oklahoma (Ruedemann, 1922) and numerous specimens indistinguishable from modern *Triops cancriformis* have been collected in Triassic formations of Virginia (Gore, 1986) and Germany (Trusheim, 1937). Two species described from the Middle Jurassic of China also resemble *T. cancriformis* (Chen, 1985). Although this fossil record represents one of the most remarkable examples of morphological stasis, it does not record historical changes in the reproductive biology or population genetics of these organisms. Our results suggest that small morphological differences may nevertheless be accompanied by profound genetic differentiation, yet relatively large morphological differences may arise between closely related forms. A key feature in the evolutionary diversification of *Triops* may be the aquisition of alternatives to gonochoric reproduction. Androdioecy (*sensu* Sassaman, 1995) and parthenogenesis allow an unrestricted opportunity for local differentiation that may inaugurate genetic diversification leading to speciation processes. The complete understanding of the evolutionary history of these organisms will undoubtedly require an integrative analysis of their zoogeography, morphological variability, reproductive mode, and genetic diversification.

Acknowledgements

Many individuals assisted materially in providing specimens used in the present study. Those responsible for collecting the samples that we have examined are listed in Appendix 1 and we thank them all. Collections at Joshua Tree National Monument were made under a permit from the National Park Service. This research was partially supported by the Agricultural Experiment Station and by the Riverside Division of the Academic Senate of the University of California.

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Appendix 1.

Localities for materials examined in the present study. Place names are indicated in bold, with site codes for localities analyzed by electrophoresis (Figure 2 and Appendix 3) indicated in parentheses. Coordinates of latitude and longitude, collector's name, and year of collection follow each locality.

CALIFORNIA – Inyo County: north end of **Eureka Valley (Eur)**, 36 km SE of Deep Springs, 37°7′ N 117°42′ W, D. Juliani, 1987. Stanislaus County: laboratory culture derived from rice fields at **Modesto**,

37°39′ N 121° W, A. Grigarick, 1989. Modesto County: rice fields at **South Dos Palos**, 36°59′ N 120°38′ W, B. Tsuikimura, 1995. Tulare County: 3.9 km N of J22 near **Pixley (Pix)** National Wildlife Refuge, 35°54′ N 119°23′ W, M. Fugate & K. Kamrath, 1987. Kern County: **Rogers Dry Lake** at Edwards Air Force Base, 34°51′ N 117°49′ W, G. Pratt, 1986. San Bernardino County: **Fords Dry Lake (Frd)**, 4 km NW of Hackberry Mountain, 35°4′ N 115°16′ W, G. Pratt, 1987; **Troy Dry Lake (Try)**, 12 km E of Newberry Springs, 34°50′ N 116°34′ W, G. Pratt, 1986; **Rabbit Dry Lake**, 4 km W Lucerne Valley on north side of CA 18, 34°28′ N 117°00′ W, G. Pratt & S. Weeks, 1987; **Soggy Dry Lake (Sog)**, 6 km N of CA 247 on Bessemer Mine Rd., 34°28′ N 116°41′ W, M. Fugate, G. Pratt, & C. Sassaman, 1986; **Silver Dry Lake**, 4km N I-15 at Baker on east side of CA 127, 35°14′ N 116°05′ W, G. Pratt, 1986; **Coyote Dry Lake,** 13 km E Twentynine Palms, 3.4 km N CA 62, 34°09′ N 113°13′ W, G. Pratt, 1986; small playa on **Indian Trail Rd (Itr)** at intersection with Indian Cove Rd, 11 km NW Twentynine Palms, 34°11′ N 116°09′ W, G. Pratt, 1986; **Mesquite Dry Lake (Mes)**, 9 km N Twentynine Palms on Mesquite Springs Rd., 34°13′ N 116° 4′W, G. Pratt, 1986. Riverside County: Joshua Tree National Monument, **Squaw Tank (Squ)**, 33°56′ N 116°05′W and **Live Oak Tank** 34°00′N 116°03′W, G. Pratt, 1988.Ford Dry Lake,30 km E **Desert Center** on I-10, 33°37′ N 115°01′W, R. Anderson, 1986; dry lake due east of the **Sheephole Mountains (Shp)**,34°11′ N 115°35′ W, G. Pratt, 1987; flood-irrigated date grove at **Valerie Jean (VaJ)**, intersection of Munro Av. and 62nd St, 33°36′ N 116°14′ W, N. Tietze, 1986. San Diego County: **Clark Dry Lake (Clk)**, 3 km N of S22, 33°15′ N 116°17′ W, M. Simovich, 1987; **Anza Borrego**, 33°16′ N 116°24′ W, M. Simovich, 1989. Imperial County: Algodones Dunes, 3 mi W of **Glamis (Glm)** on CA 78, 33°00′ N 115°08′ W, R. Velten, M. Velten & S. Morey, 1986–87; **Milpitas Wash (Mil)**, 36 km E of Glamis on CA 78, 33°15′ N 114°49′ W, S. Morey and D. Janes, 1989.

NEVADA – Lincoln County: Sand Valley, 10 km NW of **Tempiute** 37°44′ N 115°33′ W, D. Juliani, 1986; Clark County: several km S **Boulder City (Bld)** on US 95, *ca* 35°53′ N 114°056′ W, D. Wilcock, 1987.

UTAH – Washington County: 24 km SE **Hurricane (Hur)** on UT 59, 37°03′ N 113°05′ W, A. Fawcett, 1990.

ARIZONA – Apache County: **Chinle Wash (Chn)**, 11 km W US191 on south side of US160, 36°57′ N 109°42′W, M. Fugate, 1988; Cochise County: **San Simon Valley** – two adjacent ponds 6 km NNW Portal**(Wal & Dar)**, 31°56′ N 109°12′ W, 3 stock watering tanks 7 km SW Portal **(Mlh, Mll, Yco)**,31°53′ N 109°04′ W, roadside ditch 4 km SW Rodeo, nm **(Tri)**, 31°49′ N 109°05′ W; gravel quarry 25 km S Rodeo, nm **(Sht)** on E side of US 80, 31°39′ N 109°11′ W, G. Bell & M. Simovich, 1982.

COLORADO – Huerfano County: 5km W **Las Animas (LAn)** County Line on west side of CO 10, 37°45′ N 104°28′ W, M. Fugate, 1988.

NEW MEXICO – Guadalupe County: 24km W **Santa Rosa (StR)**, intersection of I-40 and Co. Rd. 4HH, 34°51′ N 104°54′ W, M. Fugate, 1987; Torrance County: 32 km E of **Clines Corners** on I-40, 34°51′ N 105°18"W, M. Fugate 1897; Hidalgo County: **Lordsburg Playa (Ldb)**, US 10 at nm 338, 32°17′ N 108°53′ W, G. Pratt, 1987; Dona Ana County: **Jornada Playa**, New Mexico State University College Ranch, 40 km NNE Las Cruces, 32°35′ N 106°50′ W, N. Zucker, 1995.

KANSAS – Scott County: White Woman Basin, near **Shallow Water (ShW)**, 7km S of KS96 on east side of US83 38°24′ N 100°56′ W, M. Fugate, 1987; Seward County: 8km N of **Liberal (Lib)** on west side of US 83, 37°05′ N 100°56′ W, M. Fugate, 1987.

TEXAS – Dallam County: 30 km N **Dalhart (Dal)** on west side of US385, 36°21′N 102°29′W, M. Fugate, 1987; **Llano** County:13.4 km E TX 16 on south side of TX 29, 30°39′ N 98°32′ W, D. Belk, 1989.

MEXICO – Baja California (Norte): **Ejido Héroes de la Indepencia**), 31° 37′ N 115° 53′ W, S. Morey, 1988; Dry lake 29 km W **Bahía de los Angeles**, 29°00′ N 113°46′ W, S. Morey, 1988.

Appendix 2. Enzyme systems and electrophoretic methods

Enzyme Names	E.C. Number	Buffer System[1]	Agar	Dilution	Locus
Acid phosphatase	3.1.3.2	2	yes	yes	*Acph-3*
Aconitate hydratase	4.2.1.3	3	yes	yes	*Acon-1*
					Acon-2
Aldolase	4.1.2.13	3	–	–	*Aldo-1*
Aspartate aminotransferase	2.6.1.1	3	–	–	*Aat-1*
					Aat-2
Aldehyde oxidase		2	–	–	*Aox*
β-Glucuronidase	3.2.1.31	2	–	–	*Gus*
Glucose-6-phosphate isomerase	5.3.1.9	1	–	–	*Gpi*
Isocitrate dehydrogenase	1.1.1.42	3	–	–	*Idh-1*
					Idh-2
Lactate dehydrogenase	1.1.1.27	2	–	yes	*Ldh*
Malate dehydrogenase	1.1.1.37	2	–	yes	*Mdh-1*
					Mdh-2
Malic Enzyme	1.1.1.40	2	–	–	*Me*
Mannose-6-phosphate isomerase	5.3.1.8	1	–	–	*Mpi*
Peptidase C	3.4.13.11	1	yes	–	*Pept-C*
Peptidase D	3.4.13.11	1	yes	–	*Pept-D*
Phosphoglucomutase	5.4.2.2	1	–	–	*Pgm*
Phosphogluconate dehydrogenase	1.1.1.44	2	–	–	*Pgdh*
Superoxide dismutase	1.15.1.1	1	–	–	*Sod*

[1] 1:LiOH system of Selander et al. (1971), 2:amino-propyl morpholine system of Clayton & Tretiak (1972) at pH of 8.5, 3: amino-propyl morpholine system at pH of 7.2

Appendix 3. Allele frequencies in North American populations of *Triops*. Populations are designated by site codes; complete names and localitites are given in Appendix 1. Loci are arranged in decreasing order of diagnostic value in separating the two species. Complete enzyme nomenclature is given in Appendix 2.

Population		Pix	Wal	Sht	Tri	Ycm	Mll	Mlh	StR	LAn	Chn	Dal	Lib	ShW	VaJ	Squ	ITr	Sog	Shp	ShW	Bld	Hur	Glm	Mil	Ldb	Eur	Sht	Clk	Dar	Try	Frd	Mes
Sample size		1	11	9	5	8	3	6	32	2	8	26	11	21	32	22	32	24	36	19	25	18	11	11	9	4	4	3	2	2	1	1
Locus & Allele																																
Acon-1	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Aat–2	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Idh–2	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	–	–	–	–	–	–	–	–	–	–	–	.05	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.95	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Acph–3	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.38	–	–	–	–	.25	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.62	1.0	1.0	1.0	1.0	.75	1.0	1.0	1.0	1.0	1.0
	3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.14	–	–	.08	.09	.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	.86	1.0	1.0	.92	.91	.98	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Idh–1	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.62	.50	1.0	.78	.85	.88	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	.38	.50	–	.22	.15	.12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Pept–C	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.36	.50	–	.35	.15	.19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	.38	.50	–	.48	.45	.29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	.26	–	1.0	.17	.40	.52	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aat–1	1	–	–	–	–	–	–	–	.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.98	1.0	.94	.98	1.0	.98	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	.06	.02	–	.02	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Appendix 3. Continued

Population Locus & Allele		Pix	Wal	Sht	Tri	Ycm	Mll	Mlh	StR	LAn	Chn	Dal	Lib	ShW	VaJ	Squ	ITr	Sog	Shp	ShW	Bld	Hur	Glm	Mil	Ldb	Eur	Sht	Clk	Dar	Try	Frd	Mes
Gus	1	–	1.0	1.0	1.0	1.0	1.0	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	–	–	–	–	–
	2	–	–	–	–	–	–	–	1.0	1.0	1.0	.98	1.0	.98	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	1.0	–	–	–	–	–	–	–	–	–	.02	–	.02	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	–	1.0	1.0	1.0	1.0	1.0
Me	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.98	.86	.83	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	.14	.17	1.0	1.0	1.0	1.0	1.0	.92	1.0	.88	1.0	1.0	1.0	1.0	1.0	1.0	–	.75	1.0	1.0
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.03	–	.03	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.05	–	.09	–	–	–	–	–	–	–	.25	–	–
Mpi	1	–	–	–	–	–	–	–	.15	–	–	.03	.09	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	.29	–	1.0	.64	.55	.64	1.0	1.0	1.0	1.0	1.0	.97	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.56	–	–	.33	.36	.25	–	–	–	–	–	.03	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–	–	.11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aox	1	–	–	–	–	–	–	–	.02	.50	.40	–	.23	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.60	.50	.60	.76	.27	.50	1.0	–	1.0	1.0	.09	1.0	.96	1.0	.09	–	1.0	–	1.0	1.0	1.0	1.0	–	1.0
	3	–	–	–	–	–	–	–	.36	–	–	.24	.50	.50	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	–	–	.91	–	.04	–	.91	1.0	–	1.0	–	–	–	–	1.0	–
	5	–	–	–	–	–	–	–	.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pgm	1	–	1.0	1.0	1.0	1.0	1.0	1.0	–	–	–	.30	.14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	1.0	–	–	–	–	–	–	.95	1.0	1.0	.70	.82	1.0	1.0	–	1.0	.05	.82	1.0	–	.83	1.0	1.0	1.0	–	1.0	1.0	1.0	–	–	1.0
	3	–	–	–	–	–	–	–	.05	–	–	–	.04	–	–	–	–	–	–	–	–	.17	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	–	.95	.18	–	1.0	–	–	–	–	1.0	–	–	–	1.0	1.0	–

Appendix 3. Continued

Population Locus & Allele		Pix	Wal	Sht	Tri	Ycm	Mll	Mlh	StR	LAn	Chn	Dal	Lib	ShW	VaJ	Squ	ITr	Sog	Shp	ShW	Bld	Hur	Glm	Mil	Ldb	Eur	Sht	Clk	Dar	Try	Frd	Mes
Pept–D	1	–	–	–	–	–	–	–	.13	–	–	.15	.25	.05	–	–	–	–	–	–	–	–	–	–	–	–	1.0	–	–	–	–	–
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.87	1.0	–	.73	.69	.84	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	–	1.0	1.0	1.0	1.0	1.0
	3	–	–	–	–	–	–	–	–	–	.62	.12	.06	.11	–	–	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	.38	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–	–	–		–	–
Acon–2	1	–	–	–	–	–	–	–	–	.25	–	.07	–	–	–	.11	–	–	–	–	.75	.22	–	.18	–	–	–	–	–	–	–	–
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.75	1.0	.93	1.0	1.0	1.0	.89	1.0	1.0	.74	1.0	.25	.78	1.0	.82	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.26	–	–	–	–	–	–	–	–	–	–	–	–	–
Sod	1	1.0	–	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	–	1.0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Aldo–1	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	–	1.0	1.0	1.0	1.0	1.0
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.0	–	–	–	–	–
Mdh–1	1	–	–	–	–	–	–	–	.02	–	–	–	–	.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.98	1.0	1.0	1.0	1.0	.98	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ldh	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.98	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	–	–	–	–	–	–	–	–	–	–	.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Pgdh	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.99	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	.01	–	–	–	–	–	–	–	–	–	–	–	–	–
Mdh–2	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Gpi	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0