

RECENT AND ANCIENT ASEQUALITY IN *TIMEMA* WALKINGSTICKSJENNIFER H. LAW¹ AND BERNARD J. CRESPI²*Behavioural Ecology Research Group, Department of Biosciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6 Canada*¹*E-mail: jhlaw@canada.com*²*E-mail: crespj@sfu.ca*

Abstract.—Determining the evolutionary age of asexual lineages should help in inferring the temporal scale under which asexuality and sex evolve and assessing selective factors involved in the evolution of asexuality. We used 416 bp of the mitochondrial COI gene to infer phylogenetic relationships of virtually all known *Timema* walkingstick species, including extensive intraspecific sampling for all five of the asexuals and their close sexual relatives. The asexuals *T. douglasi* and *T. shepardii* were very closely related to each other and evolutionarily young (less than 0.5 million years old). For the asexuals *T. monikensis* and *T. tahoe*, evidence for antiquity was weak since only one population of each was sampled, intraspecific divergences were low, and genetic distances to related sexuals were high: maximum-likelihood molecular-clock age estimates ranged from 0.26 to 2.39 million years in *T. monikensis* and from 0.29–1.06 million years in *T. tahoe*. By contrast, *T. genevieve* was inferred to be an ancient asexual, with an age of 0.81 to 1.42 million years. The main correlate of the age of asexual lineages was their geographic position, with younger asexuals being found further north.

Key words.—Ancient asexuality, phylogenetics, walkingsticks.

Received November 1, 2001. Accepted May 24, 2002.

Given the two-fold demographic advantage that asexuality confers, sexual reproduction presumably provides some short term advantages, although the nature of these benefits is as yet unclear (Maynard Smith 1978; Bell 1982; Stearns 1987; Maynard Smith 1992; Hurst and Peck 1996; Butlin et al. 1998a; West et al. 1999). Conversely, the long-term advantages of the genetic variability generated by sexual reproduction are generally accepted (Bell 1982; Hurst and Peck 1996). The twiggy phylogenetic distribution of asexuals (Bell 1982), and their apparent slow evolutionary rates for nuclear genes (Tomiuk and Loeschcke 1992; Mantovani et al. 1997; Schön et al. 1998; see also Arkhipova and Meselson 2000; Normark and Moran 2000; Welch and Meselson 2001), can be considered as evidence that most of them represent evolutionary dead ends (Maynard Smith 1978; Lynch and Gabriel 1983; Lynch et al. 1993; Rispe and Moran 2000). Ancient asexuals are a direct affront to these patterns and to most current theories of sex. Thus, understanding why ancient lineages persist will potentially help to explain why most organisms are sexual (Judson and Normark 1996; Little and Hebert 1996; Johnson and Bragg 1999; Normark 1999).

There are few undisputed examples of ancient obligate asexuals (Judson and Normark 1996; Butlin et al. 1999). The best evidence for long-lived asexuality comes from the bdelloid rotifers, organisms in which no males have been sampled, there is no evidence of sex, alternative alleles at several loci are highly divergent, and there is high morphological and genetic diversity between sublineages (Judson and Normark 1996; Welch and Meselson 2000). Darwinulid ostracods also apparently comprise asexual lineages on the order of millions of years old (Chaplin et al. 1994; Griffiths and Butlin 1995; Chaplin and Hebert 1997; Butlin et al. 1998b; Schön et al. 1998), although multiple transitions to asexuality by related sexuals (Chaplin and Hebert 1997) make robust interpretation of the ages of some lineages problematic. Phy-

logenetic or taxonomic evidence consistent with ancient asexuality can also be found among beetles (Lanteri and Normark 1995; Normark 1996), aphids (Simon et al. 1996; Normark 1999; Blackman et al. 2000), mites (Perrot-Minnot and Norton 1997), walkingsticks (Mantovani et al. 2001), clams (Ó Foighil and Smith 1995), and brine shrimp (Perez et al. 1994). However, in these cases various limitations, including incomplete sampling of extant asexuals and related sexuals (Little and Hebert 1996; Dufresne and Hebert 1997), rare sex (Hurst et al. 1992; Turgeon and Hebert 1995; Belshaw et al. 1999; Simon et al. 1999), underestimation of the number of transitions to asexuality (Harshman and Futuyma 1985; Huggall et al. 1994; Pongratz et al. 1998; Johnson and Bragg 1999; Normark 1999; Delmotte et al. 2001), and possible recent extinction of sexuals (Normark 1999), reduce the confidence with which truly ancient lack of sexuality can as yet be inferred.

Timema walkingsticks (Insecta: Phasmatoptera) are useful for studying the antiquity of asexuals since there are five described asexual species, each one with a morphologically close sexual relative (Sandoval et al. 1998). Previous phylogenetic work suggested that some *Timema* asexuals were evolutionarily long lived, dating to around the start of the latest round of glaciations approximately two million years ago (Sandoval et al. 1998). However, this phylogeny contained limited intraspecific and interspecific sampling, and two asexuals, *T. shepardii* and *T. monikensis*, as well as four recently discovered sexuals, *T. poppensis*, *T. knulli*, *T. petita*, and *T. landelsensis*, were absent from this dataset (Vickery 1993; Vickery and Sandoval 2001). Asexuality could have a more recent origin if the asexual species drove its sexual progenitors to extinction, or if a closer sexual relative has not been sampled (Sandoval et al. 1998; Normark 1999). Moreover, determining the age of parthenogens should rely on more than just genetic divergence from the closest sexual

relative (Judson and Normark 1996; Little and Herbert 1996). To make a stronger case for ancient asexuality, all extant taxa should be analyzed, with minimum dates based on divergence within a putative asexual clade (Judson and Normark 1996; Little and Herbert 1996).

MATERIALS AND METHODS

Timema Taxonomy

Timema are herbivorous stick insects that live in mountainous regions of southwestern North America (Vickery 1993; Sandoval 1994; Sandoval et al. 1998). Most species feed on chaparral vegetation. *Timema* taxonomy has relied heavily on interspecific variation in male genitalia (Vickery 1993; Vickery and Sandoval 2001), and consequently the recognition of asexual species has been based on a combination of host plant use, color, female body morphology, laboratory rearing, and a lack of males (Vickery 1993; Sandoval and Vickery 1996; Vickery and Sandoval 2001).

Each of the five asexual *Timema* species has a close morphological sexual counterpart and each is found on the same or similar host plant species as its sexual relative. *Timema douglasi* (Sandoval and Vickery 1996) had previously been paired with *T. californicum* (Sandoval et al. 1998), but a closer morphological sexual, *T. poppensis*, has since been sampled (Vickery and Sandoval 1999). *Timema shepardii*, a recently described asexual, has been paired with *T. californicum* based on similarities in body morphology, predominant coloration, and host plant use (Vickery and Sandoval 1999). *Timema genevieve* and *T. tahoe* are the asexual relatives of *T. podura* and *T. bartmani* respectively (Vickery and Sandoval 1997; Sandoval et al. 1998), and *T. monikensis* is the asexual relative of *T. cristinae* (Vickery and Sandoval 1998). The fully asexual status of *T. monikensis* is questionable because males have been sampled in *T. monikensis* populations (four males and 16 females in 1999, and males at similar frequencies in other collections). These males are capable of mating but their reproductive viability is unknown; it is not uncommon for parthenogens to occasionally produce sterile males (e. g., Soumalainen et al. 1987). All known species of *Timema* have one generation per year.

Collection and Sequencing

Timema walkingsticks were collected throughout California from their host plants using sweep nets, with the goal to accurately represent intra- and interspecific genetic variation for each sexual:asexual pair. For each collected insect, 1–3 legs were removed and dried in silica gel for DNA extraction, and bodies were kept in 75% ethanol for species identification and vouchers.

DNA was extracted using phenol-chloroform and ethanol precipitation, and polymerase chain reaction (PCR) was performed using combinations of the mitochondrial COI primers S2183 or S2195 with A2566 or A3014 (Simon et al. 1994; Law 2001). PCR product was processed using exonuclease I and shrimp alkaline phosphatase, and Big Dye Cycle Sequencing (Applied Biosystems, Foster City, CA) was used to sequence a fragment about 450 bp long. Full collection and sampling information can be found in Law (2001) and

Law and Crespi (2002), and sequences are in Genbank under AF409998–AF410151.

Phylogenetic Inference

Sequences from 168 sampled *Timema* individuals representing 19 named species were aligned by eye using the program Se-AL version 1.0 alpha 1 (Rambaut 1996), and there were no insertions or deletions in the sequenced fragment. Three outgroups were used as in Sandoval et al. (1998): the phasmids *Baculum extradentatum* and *Anisomorpha buprestoides*, and the cockroach *Blatella germanica*.

The full dataset was analyzed with neighbor joining (NJ; under the Kimura 2-parameter model) and maximum parsimony (MP; heuristic searches) in PAUP 4.0b8 (Swofford 2000). To estimate ages of asexuals, the validity of a molecular clock was tested. Because an analysis of the whole dataset was too computationally extensive under maximum likelihood (ML), the dataset was pruned to contain only sexual:asexual pairs, and ML analysis was performed separately on each pair. This pruning was consistent with the phylogenetic relationships of sexuals and asexuals as determined by NJ and MP. Each pair was appropriately rooted using a closely related sexual species. ML was performed using quartet puzzling (QP) with the program Tree-Puzzle 5.0 under an HKY model of evolution and internal nodes were assessed with QP reliability (Strimmer and von Haeseler 1996). For each sexual:asexual pair, a ML phylogenetic tree was constructed and the likelihood ratio test was used to test the assumption of a local molecular clock (Strimmer and von Haeseler 1996).

The number of independent origins of asexuality in *Timema* was determined by assessing the monophyly of each asexual taxon (*T. douglasi*, *T. shepardii*, *T. monikensis*, *T. genevieve*, and *T. tahoe*). Asexuality was assumed to be irreversible. To estimate the age of parthenogenesis in *Timema*, two measures were used. A minimum age was determined using ML branch lengths for within each asexual (i.e., to intraspecific coalescence), and a maximum age was estimated from ML distance to the node corresponding to the most recent common ancestor of the asexual and its closest sexual relative (i.e., to coalescence of the asexual with the nearest sexual). Distances were converted to ages based on an arthropod molecular clock for the mitochondrial DNA (mtDNA) COI gene of approximately 2% per million years for recently diverged lineages (Brower 1994; Juan et al. 1995, 1996; Sandoval et al. 1998).

RESULTS

Phylogenetic Inference

The maximum-parsimony tree and neighbor-joining tree were very similar in topology (Fig. 1). The main clades (referred to here as the Northern, Santa Barbara, and Southern clades) corresponded well to the geography of California (Law 2001; Law and Crespi 2002). The Northern clade contained the sexual:asexual species pairs *T. poppensis*:*T. douglasi*, and *T. californicum*:*T. shepardii*, as well as the sexual species *T. knulli*, *T. landelsensis*, and *T. petita*. The Santa Barbara clade contained the *T. cristinae*:*T. monikensis* pair, and the Southern clade contained the sexual:asexual pairs *T.*

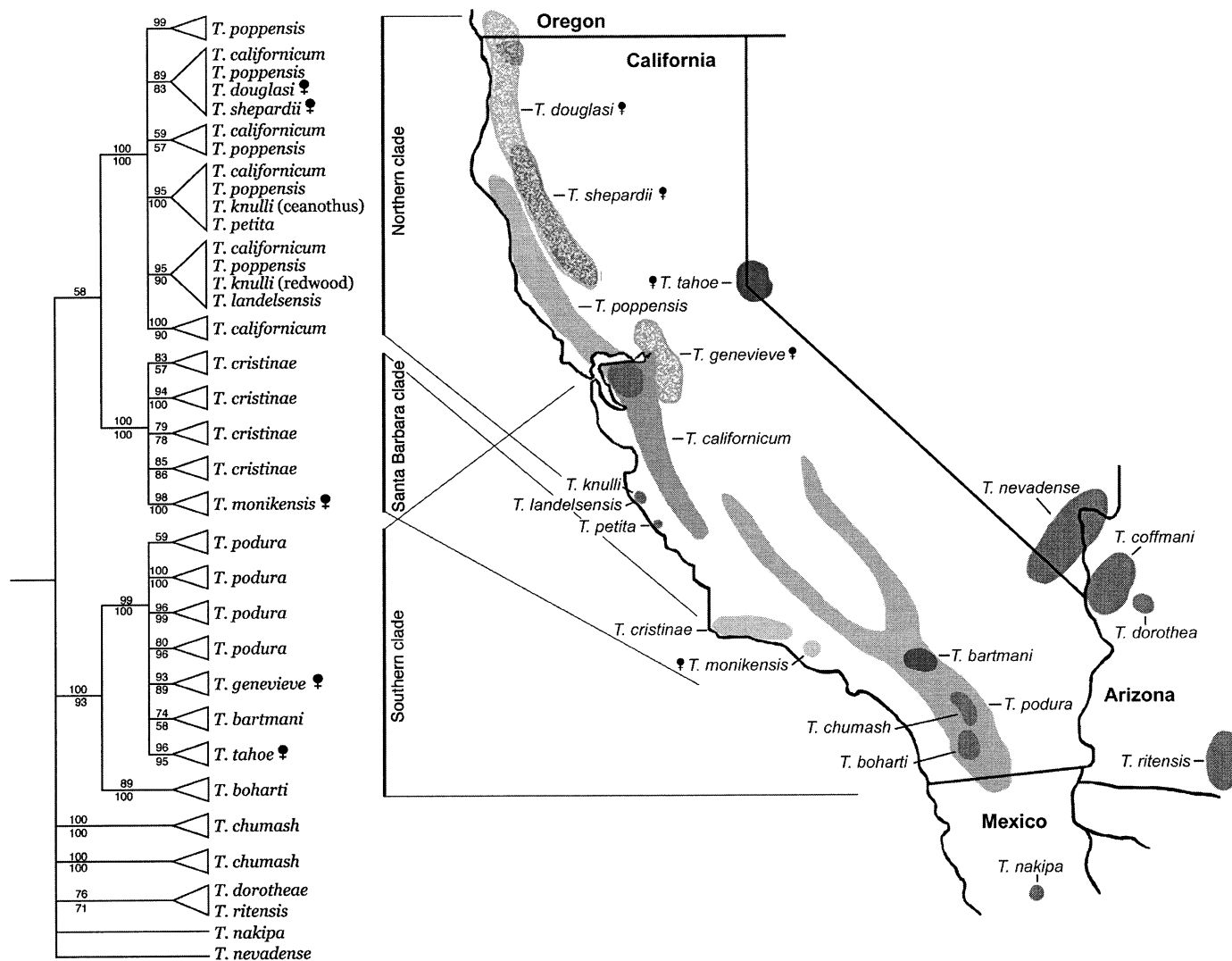


FIG. 1. Phylogenetic relationships within *Timema* inferred from neighbor-joining (NJ) and maximum-parsimony (MP) analyses of 416 bp of mitochondrial DNA (COI). All *Timema* individuals that were sequenced are not shown in this tree; rather, species positions are given within triangle tips. Asexual lineages are designated by female symbols. Numbers above branches are NJ bootstrap values (1000 replications) and numbers below show MP bootstrap values (200 replications).

podura:*T. genevieve* and *T. bartmani*:*T. tahoe*, as well as the sexual *T. boharti*.

Molecular Clock Evaluation

Within the Northern clade, *T. douglasi* and *T. shepardii* had the same or very similar haplotypes, and these haplotypes were both closely related to those of *T. poppensis*. These asexuals did not meet the criteria of monophyly. A molecular clock was rejected ($P < 0.05$) for this grouping, regardless of whether *T. shepardii* was paired with *T. californicum* (its close morphological sexual relative) or with *T. poppensis* (its closest mtDNA relative). In the Santa Barbara clade, *T. monikensis* was monophyletic and nested within its close sexual relative *T. cristinae*. A molecular clock was not rejected for the *T. cristinae*:*T. monikensis* pair ($-\log$ likelihood without clock: -1345.85 ; with clock: -1357.63 ; $\chi^2 = 23.56$; $df = 23$; $P > 0.05$), the *T. podura*:*T. genevieve* pair ($-\log$ likelihood without clock: -1220.25 ; with clock: -1239.28 ; χ^2

$= 38.08$; $df = 27$; $P > 0.05$), or the *T. bartmani*:*T. tahoe* pair ($-\log$ likelihood without clock: -749.10 ; with clock: -756.12 ; $\chi^2 = 14.03$; $df = 7$; $P > 0.05$). *Timema genevieve* and *T. tahoe* were each inferred to be monophyletic.

Age and Diversity of Asexuals

Under the assumption that asexuality cannot revert back to sex, there were four or five independent origins of asexuality in *Timema*. *Timema douglasi* and *T. shepardii*, the two parthenogens in the Northern clade, were very closely related ($0.31 \pm 0.35\%$ divergence). In fact, there was less divergence between *T. douglasi* and *T. shepardii* than within *T. douglasi* ($0.77 \pm 0.58\%$). Consequently, it is difficult to determine, using this dataset, whether these species represent independent origins. If we assume that transitions to asexuality are rare, then *T. shepardii* does not represent an independent origin of asexuality. *Timema monikensis*, *T. genevieve*, and *T. tahoe* were each monophyletic and closely related to their

respective sexual counterparts (*T. cristinae*, *T. podura*, and *T. bartmani*). Each of these lineages was inferred to contain a single, independent origin of asexuality.

In the Northern clade, both asexuals *T. douglasi* and *T. shepardii* were closely related to *T. poppensis* ($1.03 \pm 0.63\%$ divergence, and $0.98 \pm 0.64\%$ divergence, respectively). Although a molecular clock was rejected for this clade, if there is rate heterogeneity clonal ages can be roughly estimated using sequence divergence. There was so little divergence within this group that it is reasonable to infer that these asexuals are relatively young (less than 0.5 million years). If, however, *T. californicum* turns out to be the true sexual relative of *T. shepardii* ($2.57 \pm 0.78\%$ divergence), then an older age could be applied to this asexual.

The asexual *T. monikensis* exhibited a low level of intraspecific divergence ($0.63 \pm 0.41\%$) but high divergence from *T. cristinae* ($3.99 \pm 0.59\%$). Maximum-likelihood molecular clock age estimates for this clade ranged from 0.26 million years (based on intraspecific coalescence) to 2.39 million years (based on age of the most recent common ancestor of *T. monikensis* and *T. cristinae*; Fig. 2A).

The asexual *T. tahoe* also showed low intraspecific divergence ($0.51 \pm 0.25\%$). This species was $2.93 \pm 0.36\%$ diverged from its closest sexual relative, *T. bartmani*. From the maximum-likelihood molecular clock analysis, an age range of 0.29 to 1.06 million years was inferred for this asexual (Fig. 2B).

The best evidence for antiquity in *Timema* comes from the sexual:asexual pair *T. podura*:*T. genevieve*. There were two geographically and genetically distinct *T. genevieve* populations that differed from one another by an average of 2.11%, and maximum-likelihood molecular clock based age estimates for this clade ranged from 0.81 to 1.42 million years (Fig. 2C).

DISCUSSION

One of the problems with assessing antiquity of asexuals is that the term is difficult to define—it is debatable what ancient actually means in an evolutionary sense (Griffiths and Butlin 1995). Referring to *Poeciliopsis* fish, Maynard Smith (1992) stated that 100,000 years is not very long in evolutionary terms. However, Vrijenhoek (1993) pointed out that this time range means 200,000 generations in *Poeciliopsis*, and that 200,000 generations is sufficiently long for asexuals to accumulate deleterious mutations. More recently, age estimates have focused on millions of years, even though it is biologically more relevant to discuss age in terms of number of generations. For the purpose of this paper, as a conservative estimate, 500,000 generations will be considered long enough to warrant a claim of antiquity. It seems reasonable to assume that this timescale is long enough, relative to the usual lifespan of many sexual species (e.g., Hansen 1980; Kammer et al. 1998), for extinction of asexual lineages if they were evolutionary dead ends. At one generation per year, if *Timema* asexuals are ancient, then asexual lineages should form monophyletic groups that diverged from their closest sexual relatives at least 0.5 million years ago.

Timema asexuals show notable differences in inferred ages. The asexuals *T. douglasi* and *T. shepardii* appear to be quite

young (less than 0.5 million years), *T. genevieve* appears to be ancient (at least 0.81 million years) and *T. monikensis* and *T. tahoe* exhibit intermediate (though quite uncertain) ages. The evidence for antiquity, or a lack thereof, in *T. monikensis* and *T. tahoe* is weak because for each of these asexuals only one population has been found, the divergence within that population was relatively low, and both of these asexuals were substantially diverged from their sexual counterparts. Two possible explanations could account for this limited intraspecific divergence. First, the high divergence from sexuals may be due to extinction of sexual progenitors, inadequate sampling of sexuals, or a recent high extinction rate of asexual populations. Second, inadequate sampling of asexuals could lead to underestimates of their ages. Generally, specimens were collected from easily accessible locations, and several individuals were often sampled from a single plant. Spatial variation and nonrandom distribution of clone frequencies have been shown in other asexual taxa (Christensen and Noer 1986; Schenck and Vrijenhoek 1986; Hugall et al. 1994) and asexual populations may be geographically structured in such a way that limited sampling would greatly reduce the amount of detected genetic variability. Many authors agree that sampling effort can influence age estimates if the closest sexual relative is not found (Sandoval et al. 1998; Johnson and Bragg 1999; Normark 1999) but the possibility that inadequate sampling substantially reduces the amount of observed divergence within an asexual clade has largely been neglected (but see Little and Hebert 1996).

The phylogenetic distribution of asexuality versus sex has often been considered in terms of processes acting in the short term versus the long term (Moritz 1991; Griffiths and Butlin 1995; Normark 1999). Several studies have demonstrated considerable diversity in the ages of the different asexual lineages within a focal clade (Chaplin et al. 1994; Ó Foighil and Smith 1995; Normark 1996; Dufresne and Hebert 1997; Butlin et al. 1998b), but in none of these cases has the among-lineage age variation of asexuals been attributable to its putative causes. In *Timema* walkingsticks, the age of the asexual lineages appears to be associated with geography: the youngest asexuals *T. douglasi* and *T. shepardii* are found at the northern end of the range for this genus, whereas the oldest asexual, *T. genevieve*, is also the most southerly one, aside from the atypical, male-containing species *T. monikensis* (Fig. 1). This phylogeographic pattern, coupled with the evidence for geographic parthenogenesis in *Timema* and other taxa (Glesener and Tilman 1978; Bell 1982; Peck et al. 1998; Sandoval et al. 1998, Law and Crespi 2002), suggests that the recent derivation of most asexuals is due in part to their enhanced ability to colonize habitats that are recently available (e.g., more northerly, or disturbed by factors other than glaciation; Bell 1982; Bullini 1994), but also highly prone to disturbance over short evolutionary time spans (e.g., Griffiths and Butlin 1995; Dufresne and Hebert 1997).

The young age of more northerly *Timema* asexuals can largely be ascribed to glaciation-induced changes in host-plant distributions and recent colonization (Law and Crespi 2002), but in more southerly regions, ecological interactions or differences between asexual and sexual relatives may also influence lineage durations (e.g., Chaplin 1993; Moritz 1993). The two northern *Timema* parthenogens *T. douglasi* and *T.*

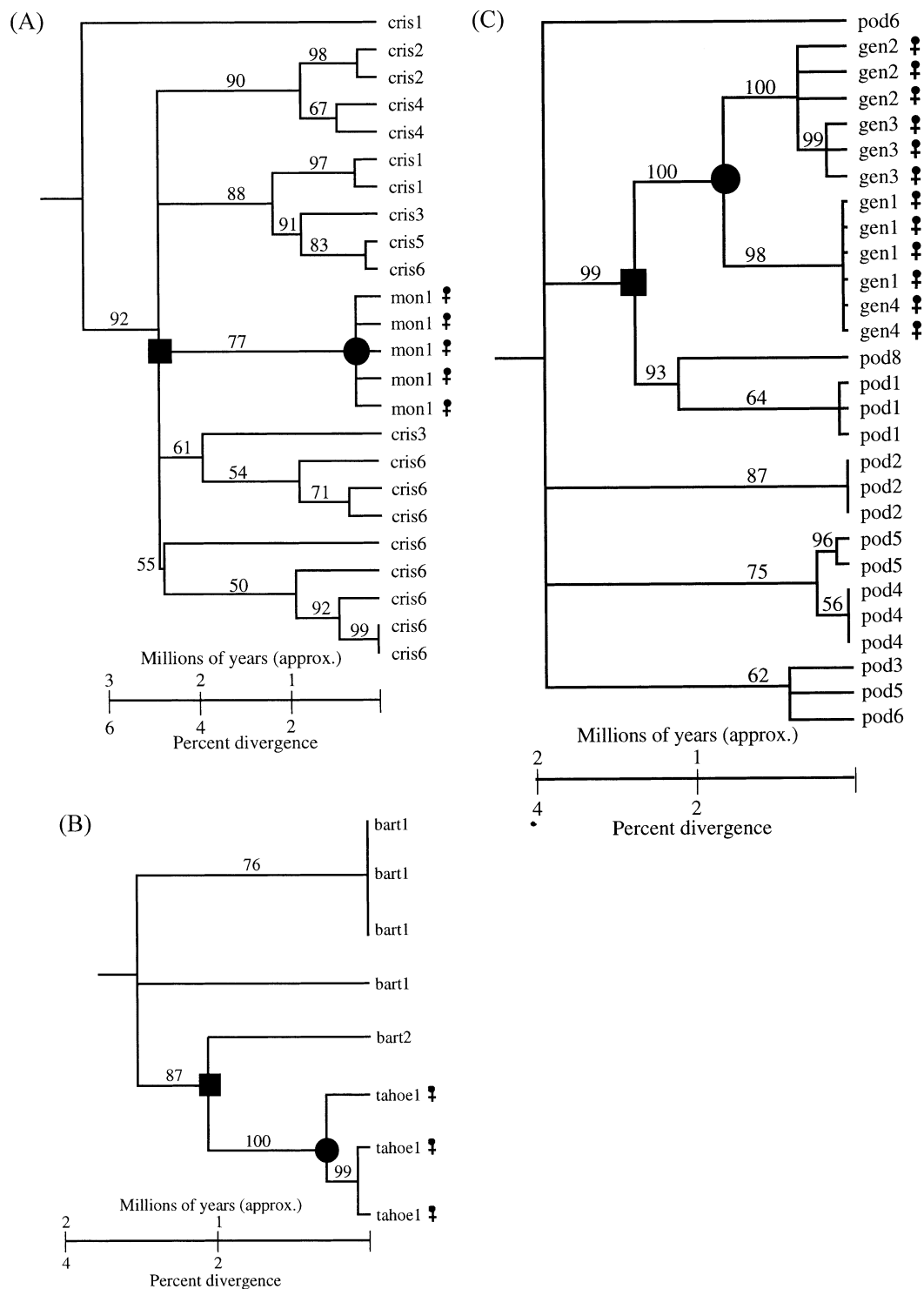


FIG. 2. Quartet puzzling (QP) maximum-likelihood trees for sexual:asexual pairs for which a molecular clock was not rejected. QP reliability indices are shown above branches. Scale bars with percent divergence and approximate ages in millions of years are given for each phylogeny. Parthenogenetic species are designated by female symbols, and numbers following species codes represent different populations (Law 2001). (A) The *Timema cristinae*:*T. monikensis* pair from the Santa Barbara clade. cris, *T. cristinae*; and mon, *T. monikensis*; cris5, AF005340 (from Sandoval et al. 1998). (B) The *T. bartmani*:*T. tahoe* sexual:asexual pair. bart, *T. bartmani*; tahoe, *T. tahoe*. The uppermost tahoe1 is AF005339 and one sample of bart1 is AF005331, both from Sandoval et al. (1998). (C) ML tree for the *T. podura*:*T. genevieve* sexual:asexual pair from the Southern clade. pod, *T. podura*; gen, *T. genevieve*. The uppermost gen3 is AF005333, and the uppermost pod5 is AF005341, both from Sandoval et al. (1998).

shepardii are geographically close to their sexual relative *T. poppensis* over a broad area in the southern part of their range, and the close proximity of these taxa suggests that competition between asexuals and sexuals may be important. By contrast, *T. genevieve*, the inferred ancient asexual, is separated from its closest sexual relative *T. podura* by more than 250 km, which suggests that *T. genevieve* has dispersed far enough to limit competition with its sexual counterpart. Thus, *T. genevieve* may also be old in part because it is geographically separated and not in direct competition with its sexual progenitor.

Our phylogenetic analysis of asexuality in *Timema* is unusually complete in that it comprises all known asexuals and almost all sexual species in the genus. Such virtually complete datasets are especially useful for testing correlates of asexuality, and for verifying the twiggy distribution of asexual lineages, which in this group is clear given the approximately 20 million year age of the genus as a whole (Sandoval et al. 1998) and the inferred presence of sexuality in all basal lineages. Moreover, the marked differences in ages among *Timema* asexuals should allow assessment of how the genetic and phenotypic evolution of asexuals differ in the short versus the long term. Further analysis of asexuality in *Timema* requires inferences from nuclear genes (Welch and Meselson 2000), elucidation of the hybrid versus tytoparthenogenetic origins of asexuality (Bullini and Nascetti 1990; Bullini 1994), and ecological/demographic studies designed to uncover the costs and benefits of abandoning sexual reproduction (Bell 1982; Maynard Smith 1992).

ACKNOWLEDGMENTS

We are grateful to M. Berbee, B. Normark, M. Smith, and C. Sandoval for helpful guidance, T. Rempel and A. Knott for field assistance, and the Natural Sciences and Engineering Research Council for financial support.

LITERATURE CITED

- Arkhipova, I., and M. Meselson. 2000. Transposable elements in sexual and ancient asexual taxa. *Proc. Natl. Acad. Sci. USA* 97: 14473–14477.
- Bell, G. 1982. *The masterpiece of nature: the evolution and genetics of sexuality*. Croom Helm, London.
- Belshaw, R. D., L. J. Quicke, W. Völkl, and H. C. Godfray. 1999. Molecular markers indicate rare sex in a predominantly asexual parasitoid wasp. *Evolution* 53:1189–1199.
- Blackman, R. L., J. M. Spence, and B. B. Normark. 2000. High diversity of structurally heterozygous karyotypes and rDNA arrays in parthenogenetic aphids of the genus *Trama* (Aphididae: Lachninae). *Heredity* 84:254–260.
- Brower, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc. Natl. Acad. Sci. USA* 91:6491–6495.
- Bullini, L. 1994. Origin and evolution of animal hybrid species. *Trends Ecol. Evol.* 9:422–427.
- Bullini, L., and G. Nascetti. 1990. Speciation by hybridization in phasmids and other insects. *Can. J. Zool.* 68:1747–1760.
- Butlin, R. K., I. Schön, and H. I. Griffiths. 1998a. Introduction to reproductive modes. Pp. 1–24 in K. Martens, ed. *Sex and parthenogenesis: evolutionary ecology of reproductive modes in nonmarine ostracods*. Backhuys, Leiden.
- . 1998b. Asexual reproduction in nonmarine ostracods. *Heredity* 81:473–480.
- Butlin, R. K., I. Schön, and K. Martens. 1999. Origin, age and diversity of clones. *J. Evol. Biol.* 12:1020–1022.
- Chaplin, J. A. 1993. The local displacement of a sexually reproducing ostracod by a conspecific parthenogen. *Heredity* 71: 259–268.
- Chaplin, J. A., and P. D. N. Hebert. 1997. *Cyprinotus incongruens* (Ostracoda): an ancient asexual? *Mol. Ecol.* 6:155–168.
- Chaplin, J. A., J. E. Havel, and P. D. N. Hebert. 1994. Sex and ostracods. *Trends Ecol. Evol.* 9:435–439.
- Christensen, B., and H. Noer. 1986. Spatial and temporal components of genetic variation in triploid parthenogenetic *Trichoniscus pusillus* (Isopoda: Crustacea). *Hereditas* 105:277–285.
- Delmotte, F., N. Leterme, J. Bonhomme, C. Rispe, and J.-C. Simon. 2001. Multiple routes to asexuality in an aphid species. *Proc. R. Soc. Lond. B* 268:2291–2299.
- Dufresne, F., and P. D. N. Hebert. 1997. Pleistocene glaciations and polyphyletic origins of polyploidy in an arctic cladoceran. *Proc. R. Soc. Lond. B* 264:201–206.
- Glesener, R. R., and D. Tilman. 1978. Sexuality and the components of environmental uncertainty: clues from geographic parthenogenesis in terrestrial animals. *Am. Nat.* 112:659–673.
- Griffiths, H. I., and R. K. Butlin. 1995. A timescale for sex versus parthenogenesis: evidence from subfossil ostracods. *Proc. R. Soc. Lond. B* 260:65–71.
- Hansen, T. A. 1980. Influence of larval dispersal and geographic distribution on species longevity in neogastropods. *Paleobiology* 6:193–207.
- Harshman, L. G., and D. J. Futuyma. 1985. The origin and distribution of clonal diversity in *Alsophila pomataria* (Lepidoptera: Geometridae). *Evolution* 39:315–324.
- Hugall, A., C. Moritz, J. Syanton, and D. R. Wolstenholme. 1994. Low, but strongly structured mitochondrial DNA diversity in root knot nematodes (Meloidogyne). *Genetics* 136:903–912.
- Hurst, L. D., and J. R. Peck. 1996. Recent advances in understanding of the evolution and maintenance of sex. *Trends Ecol. Evol.* 11: 46–52.
- Hurst, L. D., W. D. Hamilton, and R. A. Ladle. 1992. Covert sex. *Trends Ecol. Evol.* 7:144–145.
- Johnson, S. G., and E. Bragg. 1999. Age and polyphyletic origins of hybrid and spontaneous parthenogenetic *Campeloma* (Gastropoda: Viviparidae) from the southeastern United States. *Evolution* 56:1769–1781.
- Juan, C., P. Oromi, and G. M. Hewitt. 1995. Mitochondrial DNA phylogeny and sequential colonization of the Canary Islands by darkling beetles of the genus *Pimelia* (Tenebrionidae). *Proc. R. Soc. Lond. B* 261:173–180.
- . 1996. Phylogeny of the genus *Hegeter* (Tenebrionidae, Coleoptera) and its colonization of the Canary Islands deduced from cytochrome oxidase I mitochondrial DNA sequence. *Heredity* 76:392–403.
- Judson, O. P., and B. B. Normark. 1996. Ancient asexual scandals. *Trends Ecol. Evol.* 11:41–46.
- Kammer, T. W., T. K. Baumiller, and W. I. Ausich. 1998. Evolutionary significance of differential species longevity on Osagean-Meramecian (Mississippian) crinoid clades. *Paleobiology* 24: 155–176.
- Lanteri, A. A., and B. B. Normark. 1995. Parthenogenesis in the Tribe Naupactini (Coleoptera: Curculionidae). *Ann. Entomol. Soc. Am.* 88:722–731.
- Law, J. H. 2001. The evolution of geographic parthenogenesis and the persistence of asexuality in *Timema* walking-sticks. M.Sc. diss. Simon Fraser University, Burnaby, British Columbia, Canada.
- Law, J. H., and B. J. Crespi. 2002. The evolution of geographic parthenogenesis in *Timema* walking-sticks. *Mol. Ecol.*, in press.
- Little, T. J., and P. D. N. Hebert. 1996. Ancient asexuals: scandal or artifact? *Trends Ecol. Evol.* 11:296.
- Lynch, M., and W. Gabriel. 1983. Phenotypic evolution and parthenogenesis. *Am. Nat.* 122:745–764.
- Lynch, M., R. Bürger, D. Butcher, and W. Gabriel. 1993. The mutational meltdown in asexual populations. *J. Hered.* 84:339–344.
- Mantovani, B., F. Tinti, L. Bachmann, and V. Scali. 1997. The Bag320 satellite DNA family in *Bacillus* stick insects (Phas-

- matodea): different rates of molecular evolution of highly repetitive DNA in bisexual and parthenogenetic taxa. *Mol. Biol. Evol.* 14:1197–1205.
- Mantovani, B., M. Passamoni, and V. Scali. 2001. The mitochondrial cytochrome oxidase II gene in *Bacillus* stick insects: ancestry of hybrids, androgenesis, and phylogenetic relationships. *Mol. Phylogenet. Evol.* 19:157–163.
- Maynard Smith, J. 1978. *The evolution of sex*. Cambridge Univ. Press, Cambridge, U. K.
- . 1992. Age and the unisexual lineage. *Nature* 356:661–662.
- Moritz, C. 1991. The origin and evolution of parthenogenesis in *Heteronotia binoei* (Gekkonidae): evidence for recent and localized origins of widespread clones. *Genetics* 129:211–219.
- . 1993. The origin and evolution of parthenogenesis in the *Heteronotia binoei* complex: synthesis. *Genetica* 90:269–280.
- Normark, B. B. 1996. The evolution of parthenogenesis in the *Artemigus tessellatus* species complex (Coleoptera: Curculionidae): Evidence from mitochondrial DNA sequences. *Evolution* 50:734–745.
- . 1999. Evolution in a putatively ancient asexual aphid lineage: recombination and rapid karyotype change. *Evolution* 53:1453–1469.
- Normark, B. B., and N. A. Moran. 2000. Testing for the accumulation of deleterious mutations in asexual eukaryote genomes using molecular sequences. *J. Nat. Hist.* 34:1719–1729.
- Ó Foighil, D., and M. J. Smith. 1995. Evolution of asexuality in the cosmopolitan marine clam *Lasaea*. *Evolution* 49:140–150.
- Peck, J., J. M. Yearsley, and D. Waxman. 1998. Explaining the geographic distributions of sexual and asexual populations. *Nature* 391:889–892.
- Perez, M. L., J. R. Valverde, B. Batuecas, F. Amat, R. Marco, and R. Garesse. 1994. Speciation in the *Artemia* genus: mitochondrial DNA analysis of bisexual and parthenogenetic brine shrimp. *J. Mol. Evol.* 38:156–168.
- Perrot-Minnot, M.-J., and R. A. Norton. 1997. Obligate thelytoky in oribatid mites: no evidence for *Wolbachia* inducement. *Can. Entomol.* 129:691–698.
- Pongratz, N., T. F. Sharbel, L. W. Beukeboom, and N. K. Michiels. 1998. Allozyme variability in sexual and parthenogenetic freshwater planarians: evidence for polyphyletic origin of parthenogenetic lineages through hybridization with coexisting sexuals. *Heredity* 81:38–47.
- Rambaut, A. 2001. Se-AL: Sequence alignment editor Ver. 2.0. Univ. of Oxford, Oxford, U.K. Available at: <http://evolve.zoo.ox.ac.uk/software/Se-SI/main.html>.
- Rispe, C., and N. A. Moran. 2000. Accumulation of deleterious mutations in endosymbionts: Muller's ratchet with two levels of selection. *Am. Nat.* 156:425–441.
- Sandoval, C. P., 1994. The effects of the relative geographic scales of gene flow and selection on the morph frequencies in the walking-stick *Timema cristinae*. *Evolution* 48:1866–1879.
- Sandoval, C. P., and V. R. Vickery. 1996. *Timema douglasi* (Phasmatoptera: Timematodea), a new parthenogenetic species from southwestern Oregon and northern California, with notes on other species. *Can. Entomol.* 128:79–84.
- Sandoval, C., D. A. Carmean, and B. J. Crespi. 1998. Molecular phylogenetics of sexual and parthenogenetic *Timema* walking-sticks. *Proc. R. Soc. Lond. B* 265:589–595.
- Schenck, R. A., and R. C. Vrijenhoek. 1986. Spatial and temporal factors affecting the coexistence among sexual and clonal forms of *Poeciliopsis*. *Evolution* 40:1060–1070.
- Schön, I., R. K. Butlin, H. I. Griffiths, and K. Martens. 1998. Slow molecular evolution in an ancient asexual ostracod. *Proc. R. Soc. Lond. B* 265:235–242.
- Simon, C., F. Frati, A. Beckenbach, B. J. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* 87:651–701.
- Simon, J.-C., D. Martinez-Torres, A. Latorre, A. Moya, and P. D. N. Hebert. 1996. Molecular characterization of cyclic and obligate parthenogens in the aphid *Rhopalosiphum padi* (L.). *Proc. R. Soc. Lond. B* 263:481–486.
- Simon, J.-C., N. Leterme, and A. LaTorre. 1999. Molecular markers linked to breeding system differences in segregating and natural populations of the cereal aphid *Rhopalosiphum padi* L. *Mol. Ecol.* 8:965–973.
- Soumalainen, E., A. Saura, and J. Lokki. 1987. *Cytology and evolution in parthenogenesis*. CRC Press, Boca Raton, FL.
- Stearns, S. C. 1987. *The evolution of sex and its consequences*. Birkhauser Verlag, Boston, MA.
- Strimmer, K., and A. von Haeseler. 1996. Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. *Mol. Biol. Evol.* 13:964–969.
- Swofford, D. L. 2000. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4.068. Sinauer Associates, Sunderland, MA.
- Tomiuk, J., and V. Loeschcke. 1992. Evolution of parthenogenesis in the *Otiiorhynchus scaber* complex. *Heredity* 68:391–397.
- Turgeon, J., and P. N. Hebert. 1995. Genetic characterization of breeding systems, ploidy levels and species boundaries in *Cypricercus* (Ostracoda). *Heredity* 75:561–570.
- Vickery, V. R. 1993. Revision of *Timema* Scudder (Phasmatoptera: Timematodea) including three new species. *Can. Entomol.* 125:657–692.
- Vickery, V. R., and C. P. Sandoval. 1997. *Timema bartmani* (Phasmatoptera: Timematodea: Timematidae), a new species from southern California. *Can. Entomol.* 129:933–936.
- . 1998. *Timema monikensis*, species nov. (Phasmatoptera: Timematodea: Timematidae), a new parthenogenetic species in California. *Lyman Entomol. Mus. Res. Lab.* 22:1–3.
- . 1999. Two new species of *Timema* (Phasmatoptera: Timematodea: Timematidae), one parthenogenetic, in California. *J. Orthoptera Res.* 8:45–47.
- . 2001. Description of three new species of *Timema* (Phasmatoptera: Timematodea: Timematidae) and notes on three other species. *J. Orthoptera Res.* 10:53–61.
- Vrijenhoek, R. C. 1993. The origin and evolution of clones versus the maintenance of sex in *Poeciliopsis*. *J. Hered.* 84:388–395.
- Welch, D. M., and M. Meselson. 2000. Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. *Science* 288:1211–1214.
- . 2001. Rates of nucleotide substitution in sexual and anciently asexual rotifers. *Proc. Natl. Acad. Sci. USA* 98:6720–6724.
- West., S. A., C. M. Lively, and A. F. Read. 1999. A pluralistic approach to sex and recombination. *J. Evol. Biol.* 12:1003–1012.

Corresponding Editor: K. Shaw