Evolutionary Relationships of the Hawaiian and North American *Telmatogeton* (Insecta; Diptera: Chironomidae)

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ABSTRACT: Species of *Telmatogeton* and the closely related genus *Paraclunio* generally live on the rocky shores of the intertidal zone. Species of *Telmatogeton* have evolved from the marine environment into torrential freshwater streams of the Hawaiian Islands. An analysis of the banding sequences of the polytene chromosomes of species of *Telmatogeton* and *Paraclunio* from the Hawaiian Islands and North America suggests that there were at least two separate invasions from the marine to freshwater environments. One is through the marine species *T. japonicus* to an undescribed freshwater species found on east Maui. The other invasion requires a marine hypothetical species that gave rise to the Hawaiian freshwater species *T. abnormis* and *T. torrenticola*.

SPECIES OF THE FAMILY Chironomidae are generally found in aquatic or semiaquatic environments. Species in only 13 genera of chironomids, including the *Telmatogeton* and *Paraclunio*, occupy the marine environment (Hashimoto 1976, Sublette and Wirth 1980). The life cycle of most species of *Telmatogeton* and *Paraclunio* is spent on the rocky shores of the intertidal zone. In the Hawaiian Islands, species of *Telmatogeton* have evolved from the marine environment into torrential freshwater streams (Wirth 1947).

Wirth (1947, 1959) describes the discovery and naming of species of the *Telmatogeton* and *Paraclunio*. The genus *Telmatogeton* was established by J. R. Schiner in 1866, and the first species, *T. sancti-pauli*, was described by him in 1868 from specimens collected on St. Paul Island in the Indian Ocean. D. W. Coquillett described the second species, *T. alaskensis*, from specimens collected from Yakutat, Alaska, during the Harriman Expedition of 1899. The first Hawaiian freshwater species was described by F. W. Terry in 1913. J. J. Kieffer established the marine genus *Paraclunio* in 1911 for *P. trilobatus*, collected on the coast of California, and in 1915, J. R. Malloch moved *T. alaskensis* to the genus *Paraclunio*. The major morpholog-

ical feature distinguishing the genera is a sexual dimorphism of the front femur and tibia. These structures are narrow and undifferentiated in males and females of *Telmatogeton* and in females of *Paraclunio*. The front femur of *Paraclunio* males is broad, and an angular projection of the distal portion of the femur fits into a corresponding notch in the base of the tibia. Because of the morphological similarity of the two genera, the genus *Paraclunio* should be synonymized with the *Telmatogeton* (Peter Cranston, personal communication). The *Telmatogeton* and *Paraclunio* are thus essentially treated together in this discussion.

The natural history and ecology of the *Telmatogeton* and *Paraclunio* are described by Kronberg (1986), Morley and Ring (1972), and Robles (1982). Two important points relative to the current discussion are features that lead to the isolation of populations and the adaptation of species to a severe environment. Adults of both marine and freshwater species exhibit a peculiar swarming behavior; they are generally seen running about on intertidal rocks and on stream banks seeking mates. Adults are not strong fliers and thus may have limited vagility. Adults of the marine species are believed to be short-lived; they emerge on an outgoing tide, mate and lay eggs, and are washed out to sea on the incoming tide. Eggs of the marine species are laid singly in rock

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crevices, and larvae feed by grazing on the thin film of algae growing on the rock surfaces and on macroscopic green and red algae. Larvae build silky tubes from their salivary gland secretion; pupation occurs in reinforced tubes. The tubes protect larvae and pupae of the marine species from wave action at high tide and predation and desiccation at low tide, and thus allow larvae and pupae of the freshwater species to live in torrential streams and waterfalls in the Hawaiian Islands.

In 1971, Hampton Carson discovered the Hawaiian *Telmatogeton* as a material suitable for chromosome analysis (personal communication). He found the giant polytene chromosomes of the larval salivary glands to be well banded and likely to give information relative to the evolution of the freshwater species. This proved to be true, for Newman (1977) established a standard band map and proposed a provisional set of evolutionary relationships for the Hawaiian freshwater *Telmatogeton*.

All the species examined to date have polytene chromosomes suitable for detailed band analysis. Each species has six long chromosome arms with well-developed bands and a single unbanded short arm. The six long arms were designated by the letters A–F and the short arm by the letter G. A sequence of bands within arms A–F common to most of the Hawaiian freshwater species was chosen as the *Telmatogeton* standard (Newman 1977). The chromosome arms of most of the species examined have the standard sequence or differ from it by paracentric inversions. A paracentric inversion is a chromosomal mutation in which a series of bands that does not contain the centromere is rotated 180°. The specific break points of the inversion may be determined by comparing the inverted and standard band sequences. Inversions are designated by giving the arm letter of the inversion with a subscript arabic number. An inversion may be homozygous in all animals of a population or species; that is, it is fixed or it may exist along with the standard as a polymorphism. In some cases, the rearrangement of bands in a portion or even an entire arm may be so complicated by multiple inversions that it

cannot be described in terms of simple inversions. These are referred to as complex portions of an arm or complex arms.

Chromosome numbers in the *Telmatogeton* and *Paraclunio* range from $n = 7$ to $n = 3/4$ (Newman 1977). It has been proposed that the primitive number is $n = 7$ and that the other numbers are derived by centric fusions. Each centric fusion reduces the chromosome number by 1. *Telmatogeton hirtus* ($n = 3/4$) has an XY_1Y_2 sex chromosome system that may be seen in both polytene and nonpolytene chromosomes. *Telmatogeton abnormis* ($n = 4$) has an XY chromosome system that may be seen only in the polytene chromosomes. Other species do not have differentiated sex chromosomes.

Inversion sequences have been used to gain insight into possible evolutionary relationships among species. Species that share inversions are more closely related than species that do not share inversions. Carson (reviewed in Carson and Yoon 1982) used this type of analysis to determine the evolutionary relationships of over 100 species of Hawaiian *Drosophila*. Similar techniques are used here to propose a set of relationships in which there were at least two separate invasions of marine *Telmatogeton* into fresh water in the Hawaiian Islands.

MATERIALS AND METHODS

The giant banded polytene chromosomes were examined from cells of the salivary gland of fourth instar larvae fixed in acetic alcohol and stained in lactoacetic orcein. Nonpolytene chromosomes for the determination of chromosome numbers were derived from lactoacetic orcein squashes of testes and other developing larval tissues (details in Newman 1977).

RESULTS

A list of the described species of *Telmatogeton* and *Paraclunio* is given in Table 1. Band sequence and chromosome number data for all the species examined to date are given in Table 2. Included are data for an un-

TABLE 1

SPECIES OF *Telmatogeton* AND *Paraclunio*

SPECIES	GEOGRAPHIC DISTRIBUTION
Telmatogeton, marine	
T. *japonicus* Tokunaga	Japan, Hawaii,* Florida, Australia, Baltic Sea*
T. *pacificus* Tokunaga	Japan, Hawaii*
T. *pusillum* Edwards	Marquesas, Mariana, Micronesia
T. *macswaini* Wirth	California*
T. *latipennis* Wirth	Revillagigedo Islands (Pacific Ocean, Mexico)
T. *sancti-pauli* Schiner	South Africa, St. Paul Island (Indian Ocean)
T. *minor* Kieffer	South Africa
T. *australicus* Womersley	Australia
T. *antipodensis* Sublette and Wirth	Antipodes Islands (New Zealand)
T. *mortoni* Leader	New Zealand
T. *simplicipes* Edwards	South America (Ancud, Chile)
T. *trocanteratum* Edwards	South America (Ancud, Chile)
T. *atlanticum* Oliveira	South America
T. *nanum* Oliveira	South America
Telmatogeton, Hawaiian freshwater	
T. *hirtus* Wirth	Kauai*
T. *abnormis* Terry	Kauai,* Oahu
T. *fluviatilis* Wirth	Oahu*
T. *williamsi* Wirth	Oahu
T. *torrenticola* Terry	Molokai,* Maui,* Hawaii*
Paraclunio, marine	
P. *trilobatus* Kieffer	Washington,* Oregon,* California*
P. *spinosus* Hashimoto	California*
P. *alaskensis* Coquillett	Alaska,* British Columbia,* Washington,* Oregon,* California*

List of species from: Wirth (1947, 1959), Hashimoto (1976), Sublette and Wirth (1980). Species moved into the genus *Telmatogeton* by Sublette and Wirth (1980) are not included in this table.
    * Chromosomes examined.

described species from east Maui designated as *T.* new species-1 (*T.* n. sp.-1). Most of the species were discussed in Newman (1977); new data are reported here for *T. japonicus*, *T. macswaini*, the three *Paraclunio* species, and an unidentified *Telmatogeton* species from southeast Maui. Adults, required for species identification, were not collected for larvae of the southeast Maui population.

The six chromosome arms of the marine species *Telmatogeton pacificus* are totally complex relative to the *Telmatogeton* standard and thus it does not appear to be directly related to the other species. The freshwater species *T. hirtus* is largely complex relative to the *Telmatogeton* standard. Arm A differs from the standard by two fixed inversions, and the remaining arms are complex but do contain short segments that can be matched to the standard.

Examples of shared inversions appear in Figures 1 and 2. Populations of *Telmato-*

geton japonicus, *T. macswaini*, and *T. abnormis* are fixed for two inversions ($C_{3,4}$) in which inversion C_4 is included within the limits of C_3 (Figure 1). Two of the four break points appear to be at the same site in the polytene chromosome. Arm C of *T.* n. sp.-1 is complex but has one of the C_3 break points. *Telmatogeton japonicus* and *T.* n. sp.-1 share inversion E_2 (Figure 2). In addition, each species has another inversion with one break point within the limits of E_2. The proposed derivation of these chromosomes is discussed below.

Telmatogeton japonicus and *T.* n. sp.-1 share fixed inversion B_5 as well as E_2. In *T. japonicus*, arm F is standard, arms C and E differ from standard by three and two inversions, respectively, arms A and B are partly standard, and arm D is complex. For *T.* n. sp.-1, arms B, E, and F may be derived from the standard while arms A, C, and D are at least partly complex.

TABLE 2

Chromosome Band Sequences and Chromosome Numbers of *Telmatogeton* and *Paraclunio*

GENERAL LOCATION	SPECIES/SITE	n	CHROMOSOME ARM					
			A	B	C	D	E	F
Hawaii, fresh water	*T. hirtus*, Kauai	3/4	$A_{4,5}$, $A_1/+$	Complex	Complex	Complex	Complex	Complex
	T. abnormis, Kauai	4	$+$	B_2	$C_{3,4}$	$+$	$+$	$+$
	T. fluviatilis, Oahu	7	$+$	$+$	C_5	$+$	$+$	$+$
	T. torrenticola complex,							
	Molokai	4	A_2	$+$	$C_1/+$, $C_2/+$	D_4, $D_1/+$	E_1	$+$
	West Maui	7	$+$	$B_1/+$, $B_3/+$	$+$	$D_2/+$	$+$	$F_1/+$, $F_5/+$
	East Maui	6	$+$	$+$	$+$	D_5	$+$	$+$
	Hawaii (Kohala Mountains)	5	$+$	$B_7/+$	$+$	D_5	$+$	F_6
	Hawaii (Mauna Kea)	4	$B_6/+$	$+$	$+$	$D_{5.3}$	$+$	F_6
	Unidentified species, southeast Maui	7	$+$	$+$	$+$	$+$	$+$	F_8
	T. new species-1, east Maui	7	A_3, prox cx	B_5, $B_{4,8}/+$	Complex	Complex	$E_{2,3}$	$F_{2,3,4}$
Hawaii, marine	*T. japonicus*, Kauai, east Maui	7	dist $+$, prox cx	B_5, dist cx	C_7, $C_{3,4}$	Complex	$E_{2,4}$	$+$
	T. pacificus, Oahu	4	Complex	Complex	Complex	Complex	Complex	Complex
North America, marine (west coast)	*T. macswaini*, northern California	5	$+$	$+$	$C_{3,4}$, C_6	D_2, D_6	$+$	F_9
	P. trilobatus, Washington, Oregon, California	5	$+$	Complex	Complex	2/3 cx	$+$	1/2 cx
	P. spinosus, southern California	4	$+$	Complex	Complex	2/3 cx	1/4 cx	$F_{9,10}$
	P. alaskenis,* British Columbia, Washington, Oregon, California (northern form)†	5	1/2 cx, A_6	1/2 cx	Complex	2/3 cx	1/4 cx	1/2 cx

NOTE: $+$ = *Telmatogeton* standard; letter and subscript symbol = inversion; symbol/$+$ = inversion polymorphism; complex (cx) = cannot be related to standard by inversions; dist, prox = distal and proximal to centromere.

* Polymorphisms are not included in this table.

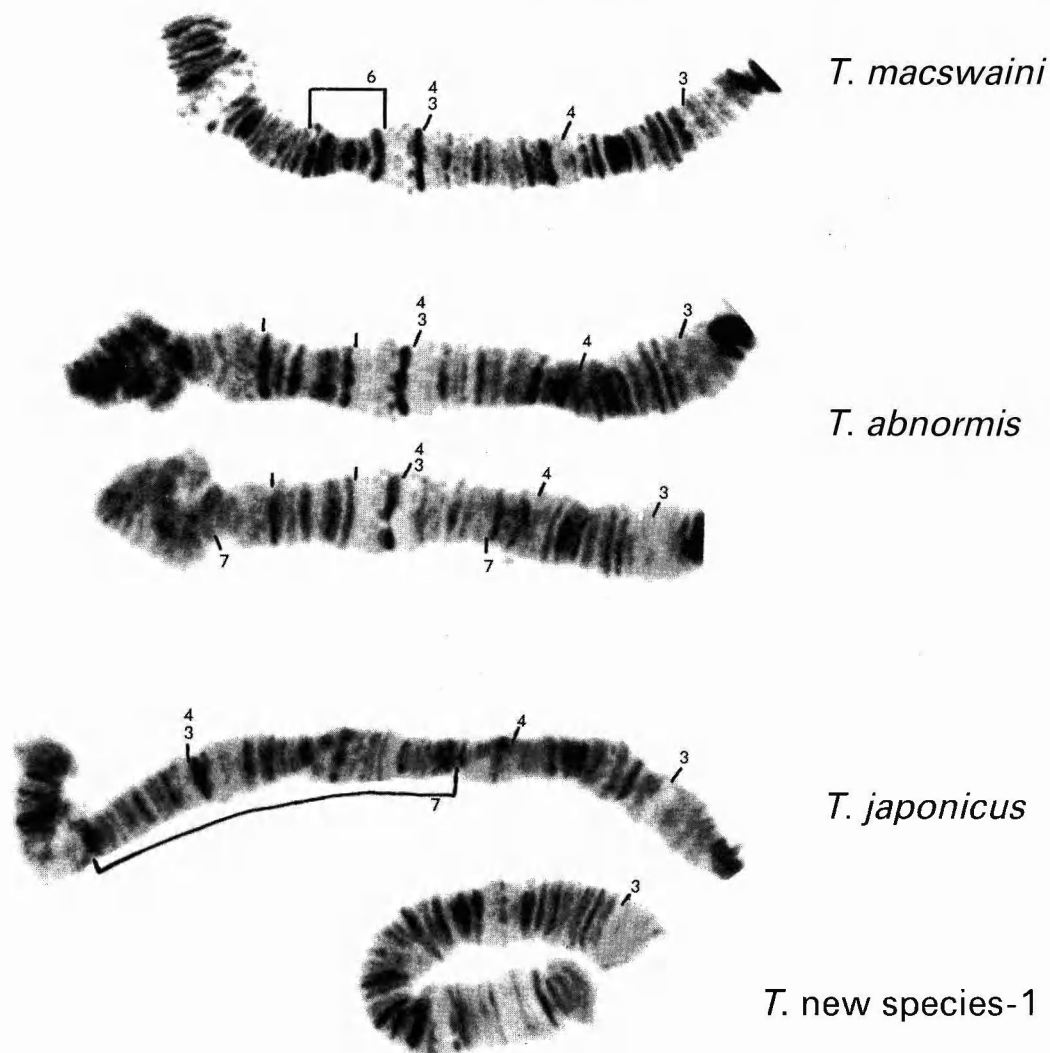† See text for explanation of northern and southern forms.

FIGURE 1. Chromosome arm C from four species of *Telmatogeton*. Inversion $C_{3,4}$ is shared by *T. macswaini*, *T. abnormis*, and *T. japonicus*. In addition, *T. macswaini* has inversion C_6 and *T. japonicus* has inversion C_7. *Telmatogeton* new species-1 has the C_3 break point proximal to the centromere. Centromeres are to the right for each chromosome arm.

The chromosomes of the remaining freshwater species have, or are close to, the standard sequence. *Telmatogeton torrenticola* is a species complex composed of five sibling species that differ by chromosome number and fixed inversions. The west Maui populations contain the standard band sequences for all chromosome arms and a chromosome number of $n = 7$. Populations to the east (on east Maui and the Kohala Mountains and Mauna Kea on Hawaii) accumulate fixed inversions and have decreased chromosome numbers. To the west, populations of *T. torrenticola* on Molokai also differ from those of west Maui by fixed inversions and reduced chromosome number (details in Table 2). *Telmatogeton fluviatilis* and the unidentified species from southeast Maui each differ from the standard by only one fixed inversion, and *T. abnormis* differs from the standard by three inversions.
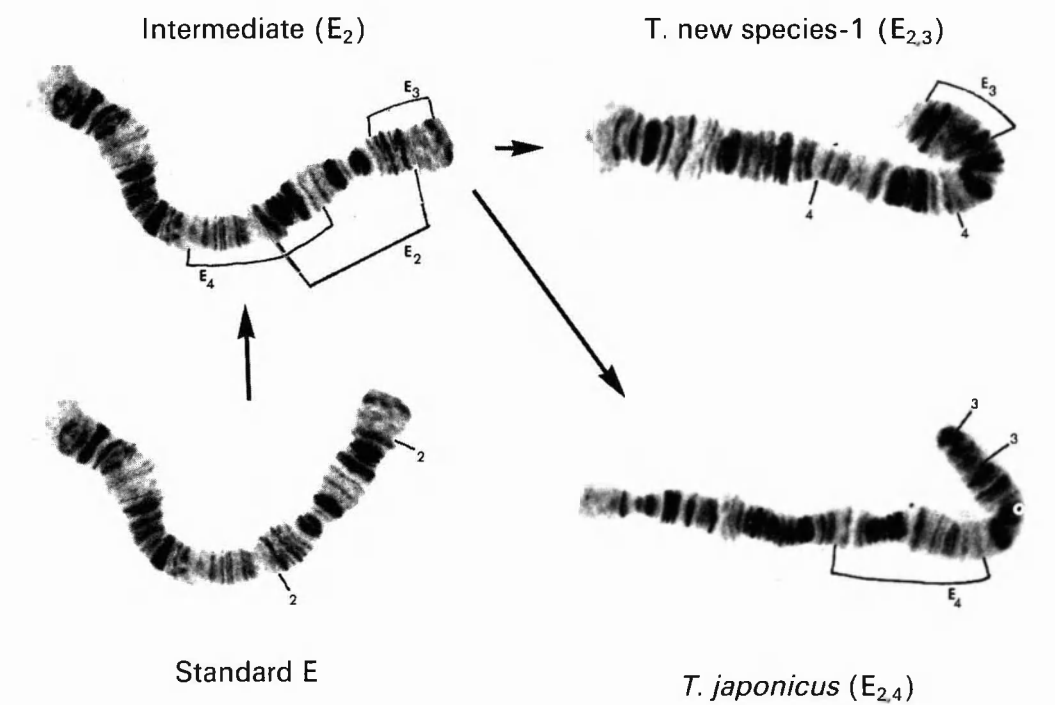
The North American marine species

FIGURE 2. Evolution of chromosome arm E in *T.* new species-1 and *T. japonicus*. The standard sequence (lower left) is considered to be primitive. Inversion E_2 is intermediate (upper left; interstitial segment of photograph in lower left cut and inverted to show inversion E_2. *Telmatogeton* new species-1 shows inversion E_3 and break points of E_4 (upper right), and *T. japonicus* shows inversion E_4 and break points of E_3 (lower right). Centromeres are to the right in each photograph.

Telmatogeton macswaini differs from the standard by six inversions in arms C, D, and F. It shares the standard sequence in arms A, B, and E with other species, inversions $C_{3,4}$ with *T. abnormis* and *T. japonicus*, and inversion D_2 with *T. torrenticola* from west Maui.

Of the three *Paraclunio* species, *P. trilobatus* is closest to the standard. It shares the standard sequence in arms A and E with other species. Arms D and F are partly standard, and arms B and C are completely complex. *Paraclunio spinosus* shares the standard sequence of arm A as well as portions of arms D and E with other species. Arm F differs from the standard by two inversions, and arms B and C are complex. Inversion F_9 is shared with *T. macswaini*.

Paraclunio alaskensis is divided into at least two distinct chromosomal forms. A northern form extends to the north from at least Yakutat, Alaska, through Moss Land-ing, California, to the south; and a southern form extends from Monterey, California, through Gaviota, California. The two forms are separated by about 30 km; hybrids between the forms have not been found. Only portions of arms A, B, D, E, and F of the northern form fit the standard sequence; the remaining segments of these arms are complex. The two forms differ from each other by a single fixed inversion in arms A and D, arms B, E, and F have the same sequences, and there are few similarities between the two forms for arm C.

DISCUSSION

Previous studies on the Hawaiian *Telmatogeton* indicated a single emergence from the marine to freshwater environments. Wirth (1947) proposed a single, unbranched

pattern of evolution with a *T. japonicus*-like species as the marine ancestor. The freshwater species evolved in the order: *T. abnormis*, *T. fluviatilis*, *T. williamsi*, *T. torrenticola*, and *T. hirtus*. His analysis was based primarily on differences in the morphology of tarsal claws in adult males.

Chromosome data (Newman 1981) also indicated *Telmatogeton japonicus* as the likely marine ancestor to the freshwater species in that it shares several band sequences with the freshwater standard. Complete data for *T. japonicus* were not available at the time because of poor material. However, *T.* n. sp.-1 was proposed as the first freshwater species because it shared the ancestral chromosome number of $n = 7$ and at least inversion B_5 with *T. japonicus*. The standard sequence, found in all arms of *T. torrenticola*

from west Maui, was then derived from the *T.* n. sp.-1 sequences by inversions. The other forms of the *T. torrenticola* complex and the remaining freshwater species were then derived from the standard sequence.

Better chromosomal data from *Telmatogeton japonicus* and an analysis of the chromosomes of the North American *Telmatogeton* and *Paraclunio* species now suggest that the *Telmatogeton* standard band sequences are for the most part ancestral rather than derived for the freshwater species other than *T.* n. sp.-1. At least two separate emergences from marine to fresh water occurred (Figure 3). One was from *T. japonicus* to *T.* n. sp.-1, and the other was through a hypothetical ancestor to *T. abnormis* and *T. torrenticola* from west Maui.

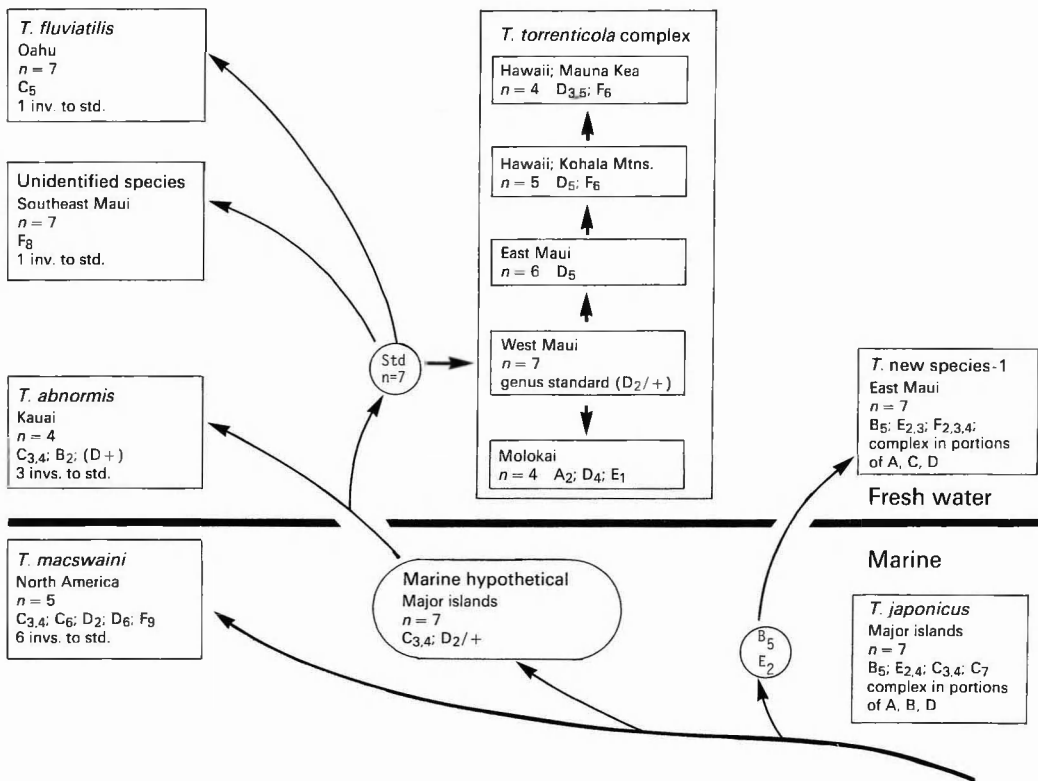It is proposed that *Telmatogeton japonicus*

FIGURE 3. Evolutionary scheme for *Telmatogeton* species from the Hawaiian Islands and North America. Intermediate chromosomal forms in circles; freshwater species above and marine species below horizontal line. See text for complete explanation.

and T. n. sp.-1 had a common ancestor in one emergence from marine to fresh water since they share inversions E_2 and B_5. Arm E of the ancestor was standard. Inversion E_2 was fixed in an intermediate species; then inversion E_3 was fixed in T. n. sp.-1 and inversion E_4 was fixed in T. *japonicus* (Figure 2). Inversion B_5, also present in the intermediate species, remained fixed in both species. Inversions $C_{3,4}$ were retained from the common ancestor in the evolution of T. *japonicus*, and additional inversions in the evolution of arm C of T. n. sp.-1 rendered that arm complex except for the C_3 break point (Figure 1). Both species retained the chromosome number of $n = 7$.

The second invasion into fresh water involves *Telmatogeton macswaini*, *T. abnormis*, and *T. torrenticola* from west Maui since the chromosomes of these species are close to the *Telmatogeton* standard sequence. A marine hypothetical species is proposed as an ancestor to the remaining freshwater species. It has the *Telmatogeton* standard band sequence with the exception of fixed inversions $C_{3,4}$ and it is polymorphic for inversion D_2. In the line from the marine hypothetical to T. *abnormis*, inversions $C_{3,4}$ are retained and inversion B_2 is fixed. The complete *Telmatogeton* standard is derived from the marine hypothetical by the inversion of $C_{3,4}$ to the standard. The D_2 polymorphism and chromosome number of $n = 7$ are retained in the evolution of *T. torrenticola* from west Maui. The chromosome numbers are reduced by centric fusions in the other sibling species of *T. torrenticola*. *Telmatogeton fluviatilis* and the unidentified species from southeast Maui are derived separately from an ancestor with the standard band sequences. The place of *T. macswaini* in the evolutionary scheme is unclear. It may have been derived from a form like the hypothetical ancestor with the fixation of four inversions, the retention of $C_{3,4}$, and the reduction in chromosome number from $n = 7$ to $n = 5$ by centric fusions. *Telmatogeton hirtus* and *T. pacificus* are not considered here since their chromosomes are so complex.

The relationships of the *Paraclunio* species to the *Telmatogeton* and even among each other is not clear. *Paraclunio trilobatus* and *P. spinosus* are closer to the *Telmatogeton* standard than is *P. alaskensis*. In *P. trilobatus*, two of the six arms are standard, and in *P. spinosus*, one arm is standard. *Paraclunio spinosus* and *T. macswaini* share inversion F_9. Placement of the *Paraclunio* species into the *Telmatogeton* phylogeny must await better analysis of the complex chromosome arms.

It is not surprising that *Telmatogeton* found its way into freshwater streams or even that multiple invasions to fresh water occurred. Wirth (1947, 1959) proposed that chironomids, which are adapted to marine environments, evolved from freshwater species because of the great competition found in the freshwater environment. In the Hawaiian Islands, where there was a lack of competition in fresh water, species of *Telmatogeton* moved back into fresh water. Limited vagility and reduction of population size or extinction of local populations caused by the severe environment may have favored the evolution of *Telmatogeton*.

At least some species of *Telmatogeton* and *Paraclunio* have retained their tie to fresh water. Wirth (1947) collected *T. japonicus* in an area of reduced salinity in the Hawaiian Islands. Tokunaga (1935) reported the development of *T. japonicus* from larvae to adults in fresh water in the laboratory. Populations of *P. alaskensis* were observed by the author to live in freshwater seepages on rock walls subject to saltwater only at high tide. Parkinson and Ring (1983) reported that larvae of *P. alaskensis* are osmoregulators and are thus able to adjust the ionic concentration of their hemolymph when living in fresh water.

The chromosome analysis of *Telmatogeton* and *Paraclunio* is incomplete. New species of *Telmatogeton* are still being discovered both in the Hawaiian Islands and other parts of the world (Sublette and Wirth 1980) and many of the described species remain to be analyzed (Table 1). However, the new data and analysis presented here brings the evolutionary scheme of the freshwater *Telmatogeton* more in line with the morphological analysis of Wirth (1947); that is, *T. abnormis* is placed close to one point of emergence

from the marine environment. New data from both morphology and cytogenetics are required for a better understanding of evolution in the genus *Telmatogeton*.

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