THE ASSOCIATION BETWEEN BREEDING SYSTEM AND
TRANSPOSABLE ELEMENT DYNAMICS IN *DAPHNIA PULEX*

A Thesis

Presented to

The Faculty of Graduate Studies

of

The University of Guelph

by

PEGAH VALIZADEH

In partial fulfilment of requirements

for the degree of

Master of Science

July, 2007

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ISBN: 978-0-494-33918-3

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ABSTRACT

THE ASSOCIATION BETWEEN BREEDING SYSTEM AND TRANSPOSABLE ELEMENT DYNAMICS IN *DAPHNIA PULEX*

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University of Guelph, 2007

Advisor:

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This thesis investigates the association between breeding system and transposable element (TE) dynamics in *Daphnia pulex*. TEs are one of the major sources of variation in genomes and *Pokey*, a class II TE, is a stable element of *Daphnia* genomes. *D. pulex* has two reproductive modes, obligate and cyclical parthenogenesis. Using a PCR based approach, TE-Display, *Pokey* number in 22 cyclic and 22 obligate isolates of *D. pulex*, and in 46 isolates from eight cyclic populations was determined. I hypothesized that the average number of *Pokey* fragments per individual would be significantly higher in cyclic than in obligate individuals and this was supported by the results, suggesting that breeding system does affect the dynamics of TEs. This study also shows two new characteristics of *Pokey*: TTAA is not the only site into which *Pokey* inserts, and *Pokey* can insert in both the reverse and forward direction in 28S rRNA genes.

To Zeineddin and all many other innocent working children around the world, whose
very basic right of education is totally ignored.

Acknowledgement

I would like to express my sincere gratitude to all those who gave me the opportunity to accomplish this thesis. This work would not be possible without the generous efforts of Angela Holliss and Elizabeth Holmes in the Genomics Facility of the University of Guelph. Many thanks to my advisor, Dr. Teresa Crease, for all her help and supervision. I would also like to thank my advisory committee members, Dr. Janet MacInnes and Dr. Denis Lynn, for their constructive advices.

Enormous amounts of deepest appreciation and admiration to my amazing parents and my beloved brother, Pouya, for providing the most loving environment. Without their infinite and generous care, affection and support, I could hardly survive the challenging period of times that has passed.

I'm truly grateful to all my friends, especially Rodrigo Leon Cordero, Irene Delgado Fernandez and Anna Moszczynska for their thoughtful support and for constantly reminding me of my abilities.

At the end, I would like to sincerely acknowledge the people of my country, Iran, who gave me the opportunity to enjoy the right of “free education” from the elementary school until the end my undergraduate studies.

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Introduction

Transposable elements

Not long ago genomes were considered to be relatively static, but it is now known that there is significant intraspecific variation among genomes (Lodish et al., 1996). Transposable elements (TEs), which are sequences that are capable of multiplying themselves in their hosts' genomes, are one of the major sources of this variation. These sequences were first discovered by Barbara McClintock in the 1940s during her studies of maize (Lodish et al., 1996). She noticed that the color patterns of kernels changed in crosses of different maize strains. She explained the results by suggesting that there are some controlling elements that regulate gene activity, which are capable of relocating from one place to another on the chromosomes. McClintock suggested that as cells divide, these elements move and make gene mutations in only some of the cells. In her studies the pigmentation genes were targeted, therefore different color patterns occurred in kernels. Her main argument was that these "regulatory elements" have major effects on the development of the organism. McClintock received the 1983 Nobel Prize for her discovery, but it took almost half a century for scientists to begin to understand how these elements function. It is now known that these elements have substantial influence on the evolution of genomes, and this effect can be through the controlling of gene activity.

During the last three decades, many studies have been done on TEs and numerous families of them have been discovered and characterized. They are present in virtually all organisms (Zhang et al., 2004) and thus, the nature of the relationship between the elements and their hosts is of considerable interest, as are the consequences TEs may have on the evolution of hosts' genomes (Kidwell and Lisch, 2001). Nevertheless, there have

been very divergent opinions about the impact of TEs on their hosts' genomes. Since TEs can generate mutations when they transpose they were considered to be molecular parasites and were called selfish or junk DNA. However, it was later revealed that TEs can have advantageous as well as harmful effects on the genomes of their hosts (Hurst and Werren, 2001). Most copies of TEs are selectively neutral and not necessarily damaging (Lodish et al., 1996). Their mutational activities can even enhance the genetic diversity of organisms within populations (Lodish et al., 1996). During evolution, recombination between the mobile elements that are dispersed throughout genomes can play a significant role in generating gene duplications and other DNA rearrangements, which contributes to the evolution of new genes (Lodish et al., 1996).

Transposition is a term which has long been used in genetics to describe the transfer of chromosomal segments from one position to the other in major structural rearrangements (Griffiths et al., 1999). When TEs move, they can have significant effects on the host: for example, they may interfere with a gene's function if they insert into coding or regulatory elements. The rate of mutation generated by insertion of TEs varies among organisms. The reason for these differences is not known, but global genomic characteristics, such as the rate of recombination may be involved (Biemont and Vieira, 2006). A unique characteristic of TEs is that they do not exploit extra-chromosomal elements, such as plasmid DNA or phage, to move around the genome. Instead, they move directly from one site to another site in the host's genome (Lewin, 1994). With the exception of non-autonomous elements that must use other TEs to move around the genome, TEs encode all the enzymes needed to move from one location in the DNA of the host and splice into another genomic location (Biemont and Vieira, 2006).

With the potential of generating genomic rearrangements, TEs may have influential regulatory roles. As they are integral components of genomes, analyzing TEs can give information not only about their own evolutionary dynamics, but also about the evolution of the host genome as well. TEs are also capable of moving between genomes of different species, a phenomenon known as horizontal transfer. This is thought to occur by vectors including viruses (Vitulo et al., 2007), parasitoid wasps, and parasitic mites (Silva et al., 2004). Thus TEs are seen as major initiators of genetic changes, which may assist species in adapting to environmental changes (Biemont and Vieira, 2006).

TEs often constitute a substantial proportion of genome size. For example, about 45% of the human genome consists of TEs (Biemont and Vieira, 2006). In fact, they make up the largest component of animal and plant genomes (Holligan et al., 2006). It is thought that TEs have gradually accumulated over evolutionary time until the transposition rate is balanced by the rate at which they are eliminated from the genomes of modern day organisms (Nuzhdin and Petrov, 2003). Therefore, the number of TEs in a species is a balance between natural selection, which decreases their copy number, and transposition, which increases their copy number. TEs are eliminated by the deletion of segments of DNA that contain them (Nuzhdin and Petrov, 2003). On the other hand, mutations occurring in the elements can often inactivate them, and then they may remain in the host genome without transposition. Eventually, mutations may accumulate in inactive TEs until they no longer appear to belong to their original TE family. Since their elimination from genomes can be very slow, TEs have accumulated during evolution to the point where they account for a large fraction of some eukaryotic genomes (Nuzhdin and Petrov, 2003). If the rate of TE increase is faster than their elimination rate, then they can persist despite

any adverse effects that they may have on host survival and reproduction (Nuzhdin and Petrov, 2003).

There is no official system for classifying TEs, but there is a simple way to categorize them based on their evolution and the genetic components they possess (Biemont and Vieira, 2006). They are generally divided into two major classes defined by their sequence organization and mechanism of transposition (Finnegan, 1990). Class I elements are typically called retrotransposons and move via RNA intermediate, which is transcribed by RNA polymerase and is subsequently converted back into DNA by a reverse transcriptase. In contrast, class II TEs, which are typically known as transposons, transpose via a DNA intermediate and replicate through a DNA based “cut-and-paste” mechanism involving a transposase protein (Hallet and Sherratt, 1997). Class II TEs typically possess either short or long terminal inverted repeats (TIR) (Finnegan, 1990). The number of TEs in each class varies in different organisms. In addition, the copy number of TEs in each family group fluctuates from several to hundreds of thousands (Nuzhdin and Petrov, 2003).

Today, all the extensive studies on TEs support McClintock’s idea that they can control gene activity within organisms (Biemont and Vieira, 2006). The more progress that is made in understanding the regulation of TE activity, the more their role in causing diverse phenotypes in their host, such as physical appearance and susceptibility to diseases, is appreciated (Biemont and Vieira, 2006).

Pokey*, a DNA transposon in *Daphnia

Pokey is a Class II TE of approximately 7 kbp in length within about 10% of the 28S ribosomal RNA (28S rRNA) genes of the cladoceran crustacean *Daphnia pulex*. Ribosomal DNA (rDNA) in metazoan animals is composed of one or more tandem arrays of a repeat unit containing the genes encoding 18S, 5.8S and 28S rRNA separated by spacers. Preliminary estimates of rDNA copy number in *D. pulex* based on quantitative PCR range from 100 to 300 copies per haploid genome (T. Crease, pers. comm.). *Pokey*, which was discovered by Sullender (1993), has 16 bp terminal inverted repeats and is flanked by 4 bp direct repeats (TTAA). Sequencing of two full-length elements from *Daphnia pulicaria* shows that *Pokey* contains a single 1.5-kb open reading frame (ORF) that codes for a putative transposase (Penton et al. 2002). It is the only transposon yet known to insert into 28S rRNA genes (Penton et al., 2004); other arthropod TEs that insert into these genes are non-LTR retrotransposons. The conserved region of the 28S rRNA gene into which *Pokey* inserts is also a “hot spot” for insertional activity of non-LTR retrotransposons. Since *Pokey* inserts into rDNA, it is exposed to the same homogenization forces, such as gene conversion and unequal crossing over, as other rDNA-specific elements and the rDNA itself (Eickbush and Eickbush, 1995). *Pokey* is a vertically inherited and stable element of *Daphnia* genomes (Penton and Crease, 2004), which is unusual as transposons usually require horizontal transfer to stay active (Kidwell and Lisch, 2001). Although *Pokey* inserts into a single site in a highly conserved region of rDNA, it also inserts into many other genomic locations (Sullender and Crease, 2001).

Transposon dynamics and breeding system

Mating systems are believed to play a significant role in determining the fate of genomic elements including TEs. Asexual genomes may be structured very differently than those of sexual species (Wright and Finnegan, 2001). In an asexual population, TEs can increase their copy number within genomes, but they are unable to settle in new genomes (Hickey, 1982). Thus, the number of TEs per individual is expected to be higher in organisms that produce sexually than asexually (Nuzhdin and Petrov, 2003). Indeed, experimental studies in yeast have shown that TE frequency is lower in asexual populations than in sexual populations (Zeyl et al., 1996). In addition, Sullender and Crease (2001) found indirect evidence that *Pockey* transposes in *D. pulex* that reproduce sexually, but not in those that lack sexual reproduction, and Wright et al. (2001) found a greater amount of variation in TE copy number between individuals in selfing populations of *Arabidopsis thaliana* than in outcrossing populations of *Arabidopsis lyrata*. The results of these studies are consistent with the theory, which suggests that TE dynamics are likely to change with a change in reproductive mode.

The evolutionary fate of TEs is in fact dependent on the survival of their host genome and the shift from sexual to asexual reproduction can have numerous evolutionary consequences. For instance, sexual and asexual populations differ in response to both short and long term selection and also in their ability to eliminate deleterious mutations of small effect (Smith, 1978). Thus, the loss of sexual reproduction in some lineages could have consequences for their long-term evolutionary potential.

Cavalier-Smith (1980) suggested that TEs are not able to spread in any deterministic way among asexual populations with strictly clonal reproduction. However, their fate would be quite different in a sexual population since they could spread reasonably rapidly due to

their ability to colonize new genomes during zygote formation in sexual reproduction. Therefore, sexual reproduction should enhance TE propagation more than asexual reproduction (Hickey, 1982). One explanation of why differences in breeding system might affect the fate of TEs is that they may modulate the strength of selection against TEs. For example, there is a greater amount of variation in TE copy number between individuals in selfing populations than outcrossing populations, which enhances the efficacy of natural selection against insertions in the former (Wright and Schoen, 1999).

Daphnia

Daphnia is one of the most widespread cladoceran crustaceans that can be found in ponds and lakes around the world. Since the evolutionary history of species in the genus *Daphnia* has been studied extensively, the genus is a good model for further evolutionary research. Molecular phylogenetic studies demonstrate that *Daphnia* is an ancient genus that presumably originated in the Mesozoic era; this idea is supported by the distribution of *Daphnia* in the fossil record (Hebert, 1995). The haploid genome of *Daphnia* contains 10 or 12 chromosomes (Beaton and Hebert, 1994) and there are no sex chromosomes (Cristescu et al., 2006). Their relatively small size, as well as the reasonable ease with which many species can be cultured in laboratory conditions, are two more characteristics that make *Daphnia* one of the main “non-classical” model organisms. Besides their evolutionary significance, *Daphnia* have also been studied by researchers in other fields. For example, they are key organisms in temperate freshwater ecosystems as major grazers on algae and an important food source for higher trophic levels, and they have also been used in toxicological studies (Cristescu et al., 2006).

Cyclical parthenogenesis is a rare mode of reproduction among animals and is only found in monogonont rotifers, aphids and cladoceran crustaceans, such as *Daphnia* (Innes and Singleton, 2000). Females of all species of *Daphnia* are able to produce two egg types: parthenogenetic summer eggs and diapausing winter eggs. Summer eggs develop immediately to generate a clone of genetically identical descendants from a single female via apomixis. These eggs, which are kept in a brood pouch within the female, are fed both by the egg yolk and also by secretions from the mother's body (Hebert, 1995). Summer eggs are produced when environmental conditions are favorable for growth. In contrast, diapausing eggs are produced during unfavorable conditions, such as short photoperiod, high water temperature, and high population density leading to low food availability (Hebert, 1978). Diapausing eggs are deposited in an ephippium, which is a protective structure modified from the female's carapace. These eggs, which can remain dormant for a very long time and can survive freezing and desiccation, and hatch when favorable conditions return, are the main dispersal mechanism of *Daphnia* (Innes and Singleton, 2000). Diapausing eggs are usually produced sexually and thus require fertilization (cyclical parthenogenesis), but they can be produced apomictically in a few *Daphnia* species (obligate parthenogenesis) (Hebert, 1995). The difference between the two sorts of diapausing eggs is that diapausing eggs produced sexually are haploid and require fertilization prior to release while apomictically produced eggs are diploid and do not require fertilization prior to release (Sullender and Crease, 2001).

In early spring, the very few *Daphnia* present in ponds are all females that either just hatched from diapausing eggs laid the previous fall or very rarely, over-wintered as adults. When the temperature warms up, these females begin to reproduce apomictically. Since an individual female is capable of producing offspring as often as every two days, a

population of *Daphnia* can increase spectacularly during the growing season. Moreover, if suitable conditions persist for an extended period, hundreds of generations of females may be produced before any males or diapausing eggs are produced.

Daphnia pulex, the species used in this research project, is the common *Daphnia* species found in most ponds and some lakes in North America, except in the subtropical and high arctic regions (Hebert, 1995). This species has great variation in body size, with mature females ranging in length between 1.1 to 3.5 mm while the males are a bit smaller. *D. pulex* has two breeding systems: some populations reproduce by cyclic parthenogenesis, while others are obligate parthenogens. Variation between the breeding systems has been seen in different geographical habitats of *D. pulex*; some of the ponds in North America contain only obligate parthenogens, while others contain cyclical parthenogens. The geographic breeding system variation shown by *D. pulex* is due to a female-limited meiosis suppressor that is inherited as a dominant factor (Innes and Hebert, 1988). The presence of both cyclic and obligate parthenogenetic reproduction in *D. pulex* makes it an ideal species with which to study the influence of breeding system on TE dynamics.

Hypotheses and objectives

The main objective of this study is to determine if the presence of sexual reproduction influences the relative number and position of *Pokey* elements in the genome of *D. pulex*. The approach I used was to compare the level of *Pokey* insertion site variation among individual *D. pulex* from both obligately and cyclically parthenogenetic populations.

Assuming that TE insertions are deleterious, it has been hypothesized that there is an impact of breeding system on TE load within individuals (Hickey, 1982). Thus, I predict that the number of *Pokey* insertions will be lower in obligate parthenogens than in cyclic parthenogens because clonal lineages with low numbers of insertions should persist longer than those with heavier *Pokey* loads. In addition, I hypothesized that the lack of transposition in obligate parthenogens should lead to lower levels of *Pokey* insertion site variation among individuals and the number of unique *Pokey* insertion sites will be higher in the cyclical parthenogens than in obligate parthenogens.

My last hypothesis is that geographical distance has an effect on the similarity of *Pokey* insertion sites among individuals. I predict that the similarity of *Pokey* insertion sites between individuals will decrease as the distance between the ponds from which individuals were sampled increases, due to the decreased potential for gene flow between populations.

Material and Methods

Daphnia pulex samples and DNA extraction

The genetically identical offspring produced apomictically by single females collected from natural populations were pooled, and then genomic DNA was extracted from them using one of three methods: phenol chloroform extraction (Crease, 1986), CTAB extraction (Doyle and Doyle, 1987), or extraction using a GenElute Mammalian Genomic DNA Miniprep kit (Sigma Aldrich, St. Louis, MO). The extractions were all done on about 10 to 30 fresh or frozen offspring from each female, weighing approximately 100 mg (wet weight). These samples will be referred to as “isolates”.

Eighty-three isolates of *Daphnia pulex*, from a range of geographical locations across North America were analyzed (Figure 1). Of these 83 isolates, 61 are cyclical parthenogens and the rest are obligate parthenogens. The 61 cyclical isolates come from 22 populations, as do the 22 obligate isolates. One isolate was sampled from each of the obligate populations and named O1 to O22. However, more than one isolate was sampled from eight of the 22 cyclically parthenogenetic populations, which are named C1 to C22. Isolates from the same population are named CX-Y where X is the population number and Y is the isolate number (Table 1). For example, C4-2 is isolate 2 from cyclic population 4, while C4-5 is isolate 5 from the same population.

The DNA samples were used to create two separate data sets: the “breeding system” and the “population genetics” data sets. The “breeding system” data set includes all 22 obligate parthenogens and one isolate from each of the 22 cyclical populations. A single isolate was chosen randomly from each of the eight cyclic populations from which multiple isolates were sampled (Table 1). The “population genetics” data set includes the

46 isolates from the eight cyclic populations from which three to seven isolates were sampled (Table 2).

Molecular Analyses

TE-Display of *Pokey* Insertion Sites

I used a PCR-based approach called TE-Display (Wright et al., 2001) to examine the abundance and distribution of the transposable element *Pokey* in the genome of *D. pulex*. This technique involves the digestion of total genomic DNA with a frequent-cutting restriction enzyme, followed by the ligation of adaptors to the digested fragments. This digestion-ligation template is then PCR amplified using a TE-specific primer and an adaptor-specific primer to generate fragments containing genomic sequences that are immediately downstream of *Pokey* insertions (Figure 2).

In order to check the quality of genomic DNA samples before performing TE-Display, I amplified a 600 bp fragment of the mitochondrial 12S rRNA gene from all samples with primers 12SF and 12SR (All primer and linker sequences are provided in Table 3. All primers and linkers were obtained from Sigma Aldrich, St. Louis, MO). The amplification reactions were performed in a PTC-100 Thermocycler (MJ Research, Waltham, MA) for 35 cycles each consisting of 30 sec denaturing at 94 °C, 90 sec annealing at 55 °C and 1 min extension at 72 °C, with a final extension of 5 min at 72 °C. The PCR products were resolved on agarose gels (0.8%) in 1X TEA (40 mM Tris-acetate, pH 8.3, 2 mM Na₂EDTA), stained with ethidium bromide, and photographed under UV light. Samples that failed to amplify with the 12S primers were eliminated from the study.

It was necessary to evaluate the amount of DNA used at each step of the TE-Display technique in order to obtain consistent results. I used 50 ng, 100 ng, 200 ng, 300

ng, 400 ng, and 500 ng of genomic DNA in the *Bfa* I digestion-ligation reactions. I assayed 0.5 μ l, 1 μ l, 2 μ l, 3 μ l, 4 μ l, and 5 μ l of the digested-ligated DNA in the primary PCR reactions, and 60 ng, 120 ng, 180 ng, and 240 ng of the primary PCR reactions as templates in the secondary PCR reactions. I evaluated digestion time of 2, 3, 4, and 5 h and two different ligation times: one hour at room temperature or overnight at 4 °C. In addition, I evaluated 25, 30, and 35 PCR cycles for the primary and secondary PCR reactions. Every other parameter was consistent. The selection criteria were based on the consistency and repeatability of the results at each step. Therefore, at each stage ten samples were tested at the same time. After each step the products were assayed on either an agarose gel or the ABI 3730 DNA Analyzer. The condition which showed the sharpest band in most of the test samples was chosen. At the end I optimized the technique by using the following conditions: 100 ng of genomic DNA (if 260/280 ratio <2) or 400 ng (if 260/280 ratio >2) for the initial *Bfa* I digestion; four hours *Bfa* I digestion time and one hour ligation time at room temperature. I used 1 μ l of digested-ligated product as the template for the primary PCR amplification; 60-70 ng of the primary PCR product as the template for the secondary PCR amplification, and 35 cycles for both primary and secondary PCR amplifications. Based on the results of this work, I digested approximately 100 ng of *Daphnia* genomic DNA with a 260/280 ratio between 1.6 and 2 with 5 units of *Bfa* I restriction enzyme (New England Biolabs, Ipswich, MA) in a total volume of 50 μ l for 5 h at 37 °C in 1X buffer (New England Biolabs, Ipswich, MA), according to the manufacturer's specifications. If the 260/280 ratio of a genomic DNA sample was ≥ 2 , indicating that there was RNA present, I digested about 400 ng of the sample. The digested DNA for both kinds of sample was then ethanol precipitated by adding 0.1 volume of 3 M NaOAc and 2 volumes of 95% ethanol. The sample was incubated for 30

min on ice and then centrifuged for 10 min at 14,000 g. After decanting the supernatant, the pellet was washed in 600 µl of 70% ethanol for 20 min at room temperature and then centrifuged for 15 min at 14,000 g. The pellet was rinsed once more by repeating the previous step, dried and then resuspended in 15 µl of DNase free water (Gibco BRL, Carlsbad, CA). The digested DNA was then ligated to 25 pmol of Forward and Reverse *Bfa*I linkers in a 20 µl reaction with 400 units of T4 DNA Ligase (New England Biolabs, Ipswich, MA) in 1x T4 DNA Ligase buffer. The reaction was incubated overnight at 4 °C.

The ligated DNA was used as the template in a primary (preselective) PCR reaction. The reaction had a total volume of 50 µl and contained 1 µl of the digested – ligated DNA sample, 1 pmol of the primer Pok6172 F, 1 pmol of the primer *Bfa*I R, 2 mM MgCl₂, 1X PCR buffer (10 mM TrisHCl pH 8.3, 50 mM KCl), 0.8 mM dNTPs and 0.5 unit of Taq DNA polymerase (New England Biolabs, Ipswich, MA). PCR reactions were performed in a PTC-100 Thermocycler (MJ Research, Waltham, MA) for 35 cycles each consisting of 30 sec denaturing at 94 °C, 90 sec annealing at 55 °C and 1 min extension at 72 °C, and a final extension of 5 min at 72 °C

I determined the DNA concentration of the primary PCR products using a Nanodrop ND-1000 Spectrophotometer. The concentration was consistently found to be 120-140 ng/µl. Approximately 60-70 ng of the primary PCR product were used as the template for a secondary (selective) PCR amplification. This reaction was carried out under the same reaction conditions as above except that the total volume was 20 µl, only 0.25 unit of *Taq* DNA polymerase (New England Biolabs, Ipswich, MA) was used, and the forward primer was Pok6464 F labeled with the fluorescent dye, HEX (Applied Biosystems, Inc., Foster City, Calif).

The secondary PCR products were resolved on an ABI 3730 DNA Analyzer (Applied Biosystems, Inc., Foster City, Calif) in the Genomics Facility at the University of Guelph. Two microlitres of each of the secondary PCR amplification products were loaded into a 96-well ABI MicroAmp plate. A size standard was prepared by mixing 4 μ l of Rox-labeled sizing ladder (Applied Biosystem, Inc., Foster City, Calif) containing fragments ranging from 35 bp to 500 bp with 750 μ l of formamide, and then adding 15 μ l to each sample in the MicroAmp plate. The samples were then denatured at 96 °C for 5 min and placed on ice for 5 min before loading them into the ABI 3730. The GeneMapper 50_POP7_template_1 run module was used to analyze the samples.

GeneMapper™ Software v3.7 (Applied Biosystems, Inc., Foster City, Calif) was used to analyze the electropherograms generated by the ABI 3730. The software estimates the size of the peaks in the electropherogram by comparing their mobility to the known sizes of the fragments in the Rox sizing ladder and then generates a list of fragments and their sizes for each sample. I only included fragments with a peak height of 100 fluorescence units in subsequent analyses. A *Pokey* profile table was made for each isolate by recording “1” if a fragment of a particular size was present and “0” when the isolate did not have the fragment (Appendix I).

To ensure that the TE-Display fragment patterns are reproducible I repeated each individual primary PCR reaction three times for each isolate. A separate secondary PCR reaction was performed from each of the triplicate primary PCR reactions and the triplicate electropherograms for each sample were compared to each other. If at least two of the triplicate primary PCR reactions showed the same fragment pattern, then they were included in the analyses. If there were not at least two similar electropherograms for any sample, I repeated the TE-Display with new triplicates. I excluded two isolates from

which no fragments were generated from the analysis under the assumption that the TE-Display reactions did not work. If there is a *Bfa* I restriction site immediately downstream of the TTAA *Pokey* insertion site, a fragment of 160 bp should be generated by TE-Display. Thus, although there were some fragments smaller than 160 bp, I only included fragments of 160 bp and larger in the analysis. The largest fragment observed was 528 bp. I used the results to generate a *Pokey* profile for each isolate indicating the presence or absence of all the insertion fragments observed.

Cloning and sequencing of TE-Display fragments:

In order to confirm that the PCR fragments generated by TE-Display are truly *Pokey* insertions, and to determine if fragments of very similar size in different isolates are the same *Pokey* insertion site, I cloned the secondary PCR products of six isolates (C4-1, C5-1, C6-7, C17, C20, O3). I chose those isolates because they represented much of the *Pokey* insertion site variation observed among individuals. Moreover, these isolates represent different populations and different breeding systems. I used the StrataClone Blunt PCR Cloning Kit (Stratagene, La Jolla, CA) following the manufacturer's specifications. I chose 48 colonies from each experiment and grew them overnight at 37°C in 1.5 ml of Luria Broth with ampicillin (200 mg/L). Plasmid DNA was isolated from these cultures using the Montage Plasmid Miniprep kit (Millipore, Billerica, MA) according to manufacturer's specifications. The final pellet was dissolved in 50 µl of water. The sizes of the plasmid inserts were estimated by amplifying them with M13F and M13R sequencing primers and then resolving them on a 0.8% agarose gel in 1x TEA buffer. The gel was stained with ethidium bromide, and the PCR products were visualized

under UV light. Eight microlitres of plasmid DNA were sequenced in a reaction containing 10 pmol of the Pok6466 F primer, 0.5 µl of Big Dye Terminator sequencing mix (Applied Biosystems, Foster City, Calif), and 1 µl of Big Dye sequencing buffer (Applied Biosystems, Foster City, Calif) in a total volume of 12 µl. The sequencing reaction was performed on a PTC-100 Thermocycler (MJ Research, Waltham, MA) for 55 cycles, each consisting of 30 sec denaturing at 96°C, 15 sec annealing at 55°C and 4 min extension at 60°C. The samples were then resolved on an ABI 3730 DNA Analyzer (Applied Biosystems, , Inc., Foster City, Calif) in the Genomics Facility at the University of Guelph. The sequences were compared to the sequences in the GenBank database using the blastn procedure (Altschul et al. 1997) to determine what the genomic flanking regions might be. BLAST searches were done on the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/>).

PCR amplification of *Pokey* from genomic DNA

To confirm the insertion of *Pokey* elements in 28S rRNA genes, I PCR amplified genomic DNA from some *D. pulex* isolates using primers Pok6466F and 28SBR to generate a ~350 bp fragment containing the 3' end of *Pokey* and the downstream flanking region of the 28S rRNA gene. Amplification using primers Pok6172F and Pok6578R, which generates a ~400 bp internal *Pokey* fragment, was also performed on the same isolates as a positive control. The amplification reactions were performed on a PTC-100 Thermocycler (MJ Research, Waltham, MA) for 35 cycles each consisting of 30 sec denaturing at 94°C, 90 sec annealing at 55°C and 1 min extension at 72°C, and a final extension of 5 min at 72 °C. The PCR products were resolved on agarose gels (0.8%) in 1X TEA, stained with ethidium bromide and photographed under UV light.

Population genetics analysis

After obtaining the *Pokey* insertion fragment profile for each of the *Daphnia* isolates, I used the software package, Arlequin version 3.1 (Excoffier et al., 2005) to partition the variation in *Pokey* insertion sites within and among isolates from different breeding systems and among populations of cyclic parthenogens. Genetic distance was calculated between all pairs of *Pokey* profiles as $d_{xy} = \sum \delta_{xy}(i)$ where $\delta_{xy}(i)$ is equal to 0 if the alleles of the *i*-th locus are identical for both profiles, and equal to 1 if they are different (Excoffier et al., 2005). The resulting distance matrix was used to draw a Neighbor Joining (NJ) tree using MEGA v3.0 software (Kumar et al., 2004). A *t*-test was used to determine if the difference in mean number of *Pokey* insertions per individual differed between obligately and cyclically parthenogenetic isolates.

Results

Cloning of TE-Display fragments

In order to ensure that the TE-Display technique identifies *Pokey* insertions, I cloned and then sequenced the secondary PCR products, which only contain the sequence downstream of the *Pokey* insertion sites, from six isolates with different *Pokey* profiles (Appendix II). Thirty-eight clones were chosen from the six experiments for sequencing. I chose at least one recombinant plasmid clone of each size from each individual as well as clones of similar size from different isolates to determine if they contain the same *Pokey* insertion sites. Three of the clones were excluded from further analysis as their sequences revealed that they did not contain an insert. The other 35 sequenced fragments are indeed *Pokey* insertions and range in size between 162 bp and 694 bp. Fragments of the same size in the electropherograms generated by the ABI 3730 DNA analyzer do represent the same *Pokey* insertion site. For example, the *Pokey* profile of isolates C6-7 and O3, which are from two different breeding systems, both include a fragment of 259 bp and their sequences are the same. The same situation was observed in C6-7 and C5-1, which are two isolates from different populations, but the same breeding system. There were cases in which two TE-Display fragments differ in size by only a couple of nucleotides and they are in fact different insertions (Appendix II).

The results of this analysis also indicate that TTAA is not the only site into which *Pokey* is inserted (Appendix II). Eight of the cloned flanking sequences (3 from O3, 2 from C4-1, 2 from C6-7, and 1 from C17) are the 28S rRNA gene with the expected TTAA insertion site (Sullender and Crease 2001). Unexpectedly, one clone from C4-1 shows that *Pokey* can insert into the 28S rRNA gene in the opposite orientation. Although

most of the other 27 clones also have a TTAA insertion site, six have ATAA, four have TTAT, and one has TGAA, TTGG, TTAC, or TTAG (Appendix II). In the only obligately parthenogenetic isolate that was analyzed, O3, *Pokey* inserted into TTAA in five out of eight clones, but in two clones it was located in TTAT and in one clone it was located in TGAA. In the clones from cyclically parthenogenetic isolates, *Pokey*'s insertion site also varies in the same isolates.

The cloned flanking sequences were compared to sequences in GenBank using blastn analysis (Altschul et al. 1997). Only a few of the *Pokey* elements are inserted in known genes, most of which are 28S rRNA genes, as expected. One flanking region matches a microsatellite sequence from *D. pulex* (O3, Accession: AC167692.2), one matches a 5S rRNA gene (C17, AJ583108.1), and one matches, albeit very poorly ($E=2.4$), a cDNA sequence (C4-1, AB228324.1) suggesting that *Pokey* may sometimes insert into protein coding genes.

Breeding system data set

The breeding system data set contains the data from 22 cyclically and 22 obligately parthenogenetic isolates. The mean number of *Pokey* insertions in cyclical isolates is $5.18 (\pm 2.24)$, with values from 1 to 10, while the mean number of *Pokey* insertions in obligate isolates is only $3.27 (\pm 2.07)$, with values from 1 to 8. Thus, on average, obligate isolates have only 65.7% as many *Pokey* insertions as cyclic isolates. The mean number of *Pokey* insertion sites is significantly higher in cyclical parthenogens than in obligate parthenogens, as predicted (t -statistic = 2.93, P [one-tail] = 0.002).

Overall, 60 different *Pokey* insertion sites were detected. The 22 obligate isolates show 31 *Pokey* insertion sites, of which 17 (55%) are found in only one obligate

individual; the 22 cyclical isolates display 50 insertion sites, of which 28 (56%) are only found in one cyclic individual. Twenty-one insertion sites (27%), including the 242 bp fragment generated by insertions in 28S rRNA genes, which are present in 37 out of 44 isolates, were observed in at least one obligate and one cyclic individual. However, more than half of the insertion sites are only present in only one or two isolates in each breeding system (Figure 3). Five isolates without *Pokey* insertions in 28S rRNA genes (denoted 242-0) and three isolates with insertions in 28S rRNA genes (denoted 242-1) were amplified with two sets of primers; Pok6172F and Pok6578R generates an internal *Pokey* fragment and the other primer set, Pok6466 and 28SBR, generates a *Pokey*/28S rRNA fragment. The three 242-1 isolates all amplified strongly with both primer sets (Figure 4). Three of the five 242-0 isolates also amplified with the Pok/28S rRNA primer pair, although not as strongly as the 242-1 controls. One 28S rRNA gene fragment from each of a 242-1 and a 242-0 isolate was sequenced in both directions, which confirmed that the expected fragment was amplified and that there is no difference in the location of *Bfa* I restriction sites in the two isolates (data not shown).

The AMOVA results for the breeding system data set show that 96.4% of the variation in *Pokey* insertion occurs within groups (cyclic and obligate), with little additional variation among groups ($F_{ST} = 0.036$, Table 4). In the NJ tree there are only a few small clusters of cyclically parthenogenetic isolates, but otherwise I did not observe any major clustering of *Pokey* insertion profiles from the same breeding system (Figure 5).

Population genetics data set

The population genetics data set contains 46 cyclical isolates from eight different populations, divided into three groups by geographic origin: Illinois, Michigan and

Ontario (Table 2). AMOVA analysis for these three groups shows that most of the variation in *Pokey* insertion sites occurs within populations (89.3%) with little additional variation among isolates within groups or among groups, although significant variation among populations within groups (6%) was observed (Table 5). Variance among groups (5%) is not significant ($P = 0.06$). Moreover, *Pokey* profiles from the same population or from populations that are geographically close to one another do not tend to cluster together on the NJ tree (Figure 6).

The 46 cyclical isolates in the population genetics data set have 58 different *Pokey* insertion sites. Twenty five sites (43%) were observed in more than one population, but the majority of the sites are found in only one or two isolates (Figure 7). The highest number of occurrences is 40 times, which is the 28S rRNA gene (242 bp) insertion. Other sites are present in most populations, but in fewer isolates. For example, sites 201 bp and 260 bp were observed in each of six populations (12 isolates), site 192 bp was observed in seven populations (18 isolates), and site 308 bp was observed in all eight populations (23 isolates).

Discussion

In obligate parthenogens, TE number increases only within genomes, but in cyclical parthenogens, TEs can increase within genomes and spread to new genomes because of meiosis. The lack of recombination between genomes in asexual organisms is the basis of the hypothesis that TEs, as important components of most genomes, should behave differently in the two different breeding systems (Hickey, 1982). The results of this study support the hypothesis that breeding system does impact *Pokey* dynamics in *D. pulex*. On average, the number of *Pokey* insertions is significantly higher in cyclical than in obligate parthenogens as predicted. Conversely, the proportion of unique *Pokey* insertion sites in the two breeding systems is very similar, and has little effect on the level of *Pokey* insertion site variation among individuals. Although 27% of insertion sites were observed in both cyclic and obligate parthenogens, the variation is high among isolates within the same breeding system. These findings do not support the hypothesis that lack of transposition in obligate parthenogens will result in lower levels of *Pokey* insertion site variation among individuals. However, they do suggest that *Pokey* does not remain in one location for long periods of time, either because *Pokey* insertions are quickly lost by natural selection or genetic drift, or because *Pokey* is very active.

The hypothesis that *Pokey* is very active is consistent with the study by Wright et al. (2001) in which they used TE-Display to determine the population dynamics of a DNA transposon, *Ac-III*, in self-pollinating *Arabidopsis thaliana* and its close outcrossing relative, *Arabidopsis lyrata*. They provided evidence that *Ac-III* has been recently active in *Arabidopsis* and observed a frequency distribution of the element in outcrossing populations (their Figure 3a) that is very similar to the one that I observed for *Pokey*

(Figures 3 and 7). This is consistent with the suggestion by Sullender and Crease (2001) that *Pokey* is active in cyclic parthenogens. However, this also raises the question of why each obligate individual has so many unique *Pokey* insertion sites if the element is not active in obligate parthenogens (Sullender and Crease, 2001). One explanation is that the obligately parthenogenetic populations are very young and are recently derived from cyclical parthenogens. This is consistent with the results of Crease et al. (1989) who analyzed population structure of *D. pulex* from Ontario and Michigan using analysis of sequence variation in mitochondrial genes. They concluded that obligate parthenogenesis has a polyphyletic origin in *D. pulex* and that obligate clones are evolutionarily young. They suggested that obligately parthenogenetic populations of *D. pulex* have existed for less time than the coalescent times for genes within the sexual species.

The NJ tree of *Pokey* profiles from cyclical and obligate isolates (Figure 5) shows that, in general, there is little clustering of profiles within each of the two breeding systems. This result does not support the suggestion that there is a more conserved *Pokey* insertion pattern within each breeding system and is consistent with the polyphyletic origin of obligately parthenogenetic clones from cyclical parthenogens.

Similarity among *Pokey* insertion profiles is not correlated to the geographical distance between populations since shared insertion sites are found in geographically distant populations. All insertion sites except one (213 bp) that were found in five or more cyclic isolates in the breeding system data set (sites 192, 201, 213, 233, 260 and 308 bp) were also found in ten or more individuals and more than one population in the population genetics data set. For example, site 308 bp was found in 23 isolates and all eight populations of the population genetics data set. Two other sites were also quite common in this data set: site 192 bp was found in 7 populations and 18 individuals while

site 260 bp (6 populations) and site 201 bp (4 populations) were found in 12 individuals (Figure 7).

It was previously reported that *Pokey* is flanked by a TTAA site (Sullender, 1993). However, in the current study *Pokey* insertions were also found in other sites. There are two explanations for this: either TTAA is not the only site into which *Pokey* can insert, or *Pokey* originally inserted into a TTAA site, but the site was subsequently mutated. The sequence of the *D. pulex* genome will soon be available and analysis of full-length *Pokey* elements from the full genome should be helpful in distinguishing between these two possibilities. *Pokey* is thought to create a target site (TTAA) duplication when it inserts (Sullender, 1993). If the same 4 nt sequence occurs at both ends of a full-length *Pokey* element, then it most likely inserted into that location originally. In contrast, a TTAA at one end of the element, but not the other is more consistent with mutation of the TTAA site after insertion.

There is no published evidence that *Pokey* can insert in the reverse direction in 28S rRNA genes, but I identified one *Pokey* insertion that was oriented in the reverse direction. It is not known if this element is active. I also expected to see some *Pokey* insertions in protein coding regions, but except in one case, the blastn analysis did not find matches to sequences that are known to encode proteins (Appendix II). In the current study, I observed some isolates from both cyclic and obligate populations that showed no 242 bp fragment in the TE-Display analysis, although the fragment was amplified with the Pok/28S primers. A change in the *Bfa* I site downstream of *Pokey*'s insertion site could have explained why I did not observe a 242 bp fragment in the TE-Display analysis even though *Pokey* is present in some copies of the 28S gene, although the cloned fragment that I sequenced did possess a *Bfa* I site in the expected location. Alternatively, the absence of

the 242 bp fragment in the TE-Display analysis could be explained if there are very few copies of *Pokey* in the 28S rRNA gene in those few isolates.

The findings of my study are consistent with experimental studies in yeast (Zeyl et al., 1996) as well as results from earlier work on *D. pulex* (Sullender and Crease, 2001). The results of both studies suggest that TE copy number is higher in organisms that reproduce sexually compared to their asexual relatives. However, in the yeast study (Zeyl et al., 1996) as well as the assessment of *Ac*-III in *Arabidopsis* (Wright et al. 2001), the number of TE insertions was compared in two different species with different breeding systems. Thus, factors other than breeding system may have caused the variable abundance of TEs in the two species. The present study does not suffer from this complication as it was performed on individuals from the same species but two different breeding systems, as was the study by Sullender and Crease (2001). The difference between my assessment and theirs is the method that I used to detect *Pokey* insertions. They used Southern blot analysis to characterize restriction fragment patterns of *Pokey* in cyclically and obligately parthenogenetic populations. They found the average number of *Pokey* insertion sites for individuals from cyclically parthenogenetic populations to be 16.6 and 15.7 for individuals from obligately parthenogenetic populations. In contrast, I found an average of 5.2 *Pokey* insertions in cyclically parthenogenetic individuals and 3.3 insertions in obligately parthenogenetic individuals using TE-Display. I scored only fragments that are 500 bp or smaller due to the limitation of the size standard that was available. In addition the TE-Display approach only detects *Pokey* insertions that match the primer sites at the 3' end of the element. If the 3' end of a *Pokey* element is degraded, the technique will fail to detect the insertion. In contrast, a 1.1 kbp probe that could bind to elements even if there are some mismatches in the target sequences was used in the

Southern blot analysis. Consequently, the Southern blot approach could detect both active and inactive *Pokey* insertions, while the TE-Display technique is more likely to detect only active *Pokey* insertions. This is an advantage of the TE-Display technique because it is more likely to exclude inactive *Pokey* insertions, which are less likely to have an affect on the host. However, Wright et al. (2001) used the same technique that I did and they observed a higher average number of *Ac*-III (which is thought to be active like *Pokey*) insertions sites per individual: 10.7 for selfing populations and 8.2 for outcrossing populations of *Arabidopsis*. The higher average number of insertions in their study may be due to the fact that they scored fragments up to 1,000 bp, or to a higher copy number of *Ac*-III than *Pokey* in general.

Although not widely used, researchers generally agree that the TE-Display technique is a highly specific and reproducible method of detecting TE insertion sites. For instance, Biedler et al. (2003) have applied the same technique to detect TE insertion sites in individuals of the African malaria mosquito, *Anopheles gambiae* and its sibling species *Anopheles arabiensis*. They suggested that TE-Display provides powerful dominant and co-dominant genetic markers to study the behavior of TEs in mosquito populations and to illustrate the complex population genetics of the TEs they assayed. Their results revealed high levels of TE polymorphism in *A. gambiae* and although some insertions are shared between the two sibling species, many of them are unique. However, it was not clear to Beidler et al. (2003) whether the difference in TE-Display banding patterns is due to insertion site variation in the two species or to differences in the flanking sequences between them, since they are two different species. As did I, they confirmed the specificity of TE-Display by cloning and sequencing several of the fragments that they amplified using the technique.

There are some ways to improve the TE-Display technique. The improvement I would suggest is to use a size standard with larger fragments to allow detection of more *Pokey* insertions. A size standard with fragments as large as 1,000 bp is now available. Fragments larger than that would be difficult to resolve on the ABI 3730 DNA analyzer. It would also be interesting to measure the actual transposition rate in cyclically parthenogenetic *D. pulex*. This could be done by generating *Pokey* profiles from the offspring of a single female. It would be necessary to produce apomictic daughters for several generations and then allow the individuals of this clone to produce ephippial eggs and males to obtain sexually produced offspring. Transposition is detected as the appearance of a new insertion site in the *Pokey* profiles. However, hatching a large number of ephippial eggs in the laboratory may be challenging.

Conclusions

Overall, this study suggests that breeding system does indeed affect the dynamics of TEs; there are more *Pokey* insertion sites in cyclically parthenogenetic than in obligately parthenogenetic *D. pulex*. In addition, it appears that *Pokey* is active and constantly moves to new locations so that patterns of insertion sites among individuals are not correlated with the breeding system or the geographical location of the host populations. This finding supports the suggestion of Crease et al. (1989) that obligately parthenogenetic populations of *D. pulex* were very recently derived from cyclical parthenogens. The results of this study also show that TTAA may not be the only site into which *Pokey* is able to insert. Either *Pokey* is able to insert into other sites or a TTAA site into which *Pokey* originally inserted could have mutated. The current report also shows that *Pokey* can locate in the reverse direction in the 28S rRNA gene. In general, my study has provided some new insight into the dynamics of *Pokey* in two different reproductive modes. There are still many unknowns about TEs, a very important component of genomes, which demands that researchers continue to study them.

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Table 1. Number of *Pokey* insertions in 22 cyclically and 22 obligately parthenogenetic isolates of *Daphnia pulex* from eastern Canada and the United States. Source column indicates the researcher from which the DNA from each isolate was obtained. The state or province of origin of the isolates is listed. The 22 obligately parthenogenetic isolates, O1 to O22 come from 22 populations, as do the 22 cyclically parthenogenetic isolates, C1 to C22. When more than one isolate was sampled from cyclically parthenogenetic populations a single isolate was chosen randomly from each. For example C2-6 is isolate 6 which was randomly chosen from seven isolates sampled from population C2.

Isolate code	Breeding system	State/Province	Source	Number of <i>Pokey</i> insertions
C1	Cyclical	IL	Crease	9
C2-6	Cyclical	IL	Crease	6
C3-7	Cyclical	IL	Crease	10
C4-3	Cyclical	IL	Crease	7
C5-6	Cyclical	MI	Crease	4
C6-1	Cyclical	MI	Crease	3
C7	Cyclical	MI	Crease	5
C8-3	Cyclical	MI	Crease	3
C9	Cyclical	MI	Lynch	4
C10-1	Cyclical	ON	Crease	7
C11-1	Cyclical	ON	Crease	1
C12	Cyclical	ON	Crease	5
C13	Cyclical	ON	Crease	3
C14	Cyclical	ON	Lynch	3
C15	Cyclical	IN	Lynch	5
C16	Cyclical	IN	Lynch	4
C17	Cyclical	OR	Lynch	5
C18	Cyclical	MN	Lynch	5
C19	Cyclical	SK	Crease	5
C20	Cyclical	MB	Crease	9
C21	Cyclical	WI	Lynch	4
C22	Cyclical	IA	Crease	7
O1	Obligate	ME	Lynch	2
O2	Obligate	MI	Crease	3

Table 1 cont'd

O3	Obligate	MI	Lynch	7
O4	Obligate	MI	Lynch	5
O5	Obligate	MI	Innes	2
O6	Obligate	MI	Lynch	8
O7	Obligate	MI	Innes	4
O8	Obligate	MI	Lynch	1
O9	Obligate	MN	Lynch	2
O10	Obligate	NB	Lynch	3
O11	Obligate	NS	Weider	2
O12	Obligate	NY	Lynch	1
O13	Obligate	ON	Lynch	1
O14	Obligate	ON	Crease	2
O15	Obligate	QC	Crease	3
O16	Obligate	QC	Crease	8
O17	Obligate	QC	Lynch	4
O18	Obligate	QC	Lynch	2
O19	Obligate	WI	Lynch	3
O20	Obligate	MI	Lynch	3
O21	Obligate	ON	Crease	2
O22	Obligate	ON	Crease	4

Table 2. Number of *Pokey* insertions in 46 isolates from eight cyclically parthenogenetic populations of *Daphnia pulex* from Illinois (IL), Michigan (MI) and Ontario (ON). The state or province of origin of the isolates is listed. CX-Y is isolate number X from population number Y. All DNA samples were obtained from T. Crease.

Isolate code	State/Province	Number of <i>Pokey</i> insertions
C2-1	IL	1
C2-2	IL	1
C2-3	IL	3
C2-4	IL	6
C2-5	IL	1
C2-6	IL	6
C2-7	IL	1
C3-1	IL	4
C3-2	IL	1
C3-3	IL	2
C3-4	IL	7
C3-5	IL	12
C3-6	IL	9
C3-7	IL	10
C4-1	IL	8
C4-2	IL	6
C4-3	IL	7
C4-4	IL	1
C4-5	IL	9
C4-6	IL	5
C4-7	IL	4
C5-1	MI	10
C5-2	MI	3
C5-3	MI	6
C5-4	MI	4
C5-5	MI	3
C5-6	MI	4
C6-1	MI	3
C6-2	MI	4

Table 2 cont'd

C6-3	MI	1
C6-4	MI	1
C6-5	MI	11
C6-6	MI	9
C6-7	MI	7
C8-1	MI	5
C8-2	MI	6
C8-3	MI	3
C8-4	MI	4
C8-5	MI	7
C10-1	ON	7
C10-2	ON	8
C10-3	ON	2
C10-4	ON	6
C11-1	ON	1
C11-2	ON	1
C11-3	ON	4

Table 3. Sequences of the primers and the linkers used in TE-Display to generate a *Pockey* insertion profile. All oligonucleotides were purchased from Sigma Aldrich (St. Louis, MO).

Primer	Sequence
Pok6172F	5'-tggtc gat ggtaa agac c tca acg tc
Pok6578R	5'-gat gg t cgg att c gat t ga at g ct cg
Pok6464F	5'-tg g cca a a c ac g g t t t g g cc g
Pok6466F	5'-g cc gaa ac g c g g t ta g g cc g
28SBR	5'-cgt ct ccc act t at g ct ac ac ct c
<i>Bfa</i> IR	5'-gac gat gag t c ct gag tag
<i>Bfa</i> I linkerF	5'-tact c ag g act cat
<i>Bfa</i> I linkerR	5'-gac gat gag t c ct gag
12SF	5'-gt ct a acc g c g ac g g ct g g ca c
12SR	5'-cc ag ta ca t ct act t t g tt ac g ac

Table 4. AMOVA of *Pokey* insertion site variation in 22 cyclically and 22 obligately parthenogenetic isolates of *Daphnia pulex* from eastern Canada and the United States. The significance tests were based on 1,023 permutations. d.f. = degrees of freedom.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
Among groups	1	5.77	0.12	3.63	0.00098
Within groups	42	132.63	3.16	96.37	
Total	43	138.40	3.28		
F_{st}	0.036				0.00098

Table 5. AMOVA of *Pokey* insertion site variation in eight cyclically parthenogenetic populations of *Daphnia pulex* from Illinois, Michigan and Ontario. The significance tests were based on 1,023 permutations. d.f. = degrees of freedom

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	p value
Among groups	2	13.19	0.16 Va	4.75	0.06
Among populations within groups	5	21.80	0.21 Vb	5.96	0.01
Within population	38	119.10	3.13 Vc	89.30	<0.0001
Total	45	154.09	3.50981		
Fixation Indices					
F_{sc}	0.062	Among populations within groups			0.01
F_{ct}	0.047	Among groups			0.06

Figure 1. A map showing the locations from which *Daphnia pulex* isolates were collected from eastern Canada and the United States. The circles show the locations from which cyclically parthenogenetic isolates were sampled and the squares show the locations from which obligately cyclically parthenogenetic isolates were sampled. The numbers close to each circle and square show the number of isolates collected from each location.

Figure 2. A schematic view of the TE-Display technique. Genomic DNA is digested with a restriction enzyme (*BfaI*), then linkers (*BfaI* Linker R and *BfaI* Linker F) are ligated to the digested fragments. Two PCR amplifications, primary and secondary, are performed on the digested-ligated DNA. The primary PCR uses a primer that binds to the 3' end of the TE (*Pok6172 F*) and a primer that binds to the R linker (*BfaIR*). The secondary PCR products, are generated with the same reverse primer (*BfaIR*) and a dye-labeled primer (*Pok6464 F) that binds slightly downstream of *Pok6172 F*. The labeled fragments were resolved on an ABI 3730 DNA Analyzer. The box in the middle shows the location of the linkers after ligation.

Figure 3. Frequency distribution of 60 *Pokey* insertions in 22 cyclically and 22 obligately parthenogenetic isolates of *Daphnia pulex* from eastern Canada and United States.

Figure 4. Ethidium bromide stained gel of PCR amplification products from eight isolates of *Daphnia pulex*. The upper row is amplified with Pok6466F and 28SBR, while the lower row is amplified with Pok6172F and Pok6578R. N = the negative control; L = 300 bp – 10,000 bp size ladder (Norgen, St. Catharines, ON). The samples were loaded in the same order in the upper and lower rows. The loading order in both rows is as follows: C2-1, C14, C7, empty well, C6-4, O2, C5-3, C5-1, and O21. Only the first three isolates show the 28S rRNA *Pokey* insertion in the TE-Display analysis.

Figure 5. Neighbor-Joining tree of *Pokey* insertion profiles from 22 cyclically parthenogenetic (C) and 22 obligately parthenogenetic (O) isolates of *Daphnia pulex* from eastern Canada and the United States. For details of isolates, see Table 1.

Figure 6. Neighbor-Joining tree of *Pokey* insertion profiles of *Daphnia pulex* sampled from eight cyclically parthenogenetic populations in Illinois, Michigan and Ontario. For details of isolates, see Table 2.

Figure 7. Frequency distribution of 58 *Pokey* insertions in 46 cyclically parthenogenetic isolates of *Daphnia pulex* from eight populations in Illinois, Michigan and Ontario.

Appendix I Pokey profile for each isolate of <i>Daphnia pulex</i> . The number at the top of each column is the size in base pairs of each TE-Display fragment																						
Iso. #	160	162	163	165	166	171	172	175	179	182	185	186	190	192	194	195	197	201	207	212	213	
C 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	
C 2-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 2-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 2-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 2-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 2-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 2-6	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	
C 2-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 3-1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 3-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
C 3-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 3-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	1	0	
C 3-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 3-6	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	
C 3-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	
C 4-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
C 4-2	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	
C 4-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	
C 4-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
C 4-5	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	1	0	0	
C 4-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
C 4-7	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	
C 5-1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	
C 5-2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	
C 5-3	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	1	

Iso. #	160	162	163	165	166	171	172	175	179	182	185	186	190	192	194	195	197	201	207	212	213
C 5-4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
C 5-5	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
C 5-6	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
C 6-1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
C 6-2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 6-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 6-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 6-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
C 6-6	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0
C 6-7	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0
C 7	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
C 8-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 8-2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
C 8-3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
C 8-4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
C 8-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
C 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
C 10-1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
C 10-2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
C 10-3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
C 10-4	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
C 11-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 11-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 11-3	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
C 12	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
C 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

Iso. #	160	162	163	165	166	171	172	175	179	182	185	186	190	192	194	195	197	201	207	212	213
C 14	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
C 15-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 15-2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
C 16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 18	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 19	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
C 20	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 21	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
C 22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
O 1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
O 3	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 4	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
O 5	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 6	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0
O 7	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 9	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 14	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 15	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 16	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Iso. #	160	162	163	165	166	171	172	175	179	182	185	186	190	192	194	195	197	201	207	212	213
O17	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O18	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O19	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
O20	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O21	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	3	9	5	6	1	2	2	1	2	3	2	7	1	23	2	7	1	14	3	1	9

Appendix Fragment. "1" indicates the presence and "0" indicates the absence of each fragment.																							
Iso. #	214	217	220	223	230	232	233	234	236	242	245	246	248	251	253	258	260	261	264	265	268		
C 1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0		
C 2-1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 2-2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 2-3	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 2-4	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0		
C 2-5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0		
C 2-6	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 2-7	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 3-1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 3-2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 3-3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 3-4	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0		
C 3-5	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0		
C 3-6	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0		
C 3-7	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0		
C 4-1	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0	0	1		
C 4-2	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 4-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0		
C 4-4	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 4-5	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0		
C 4-6	0	0	0	0	0	0	1	1	0	1	0	1	0	0	0	0	0	0	0	0	1		
C 4-7	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0		
C 5-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0		
C 5-2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0		
C 5-3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0		

Iso. #	214	217	220	223	230	232	233	234	236	242	245	246	248	251	253	258	260	261	264	265	268
C 5-4	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
C 5-5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 5-6	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 6-1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 6-2	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0
C 6-3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 6-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 6-5	1	0	1	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0
C 6-6	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0
C 6-7	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0
C 7	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 8-1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
C 8-2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
C 8-3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 8-4	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 8-5	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
C 9	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 10-1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0
C 10-2	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0
C 10-3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 10-4	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 11-1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 11-2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 11-3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Iso. #	214	217	220	223	230	232	233	234	236	242	245	246	248	251	253	258	260	261	264	265	268
C 14	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0
C 15-1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0
C 15-2	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
C 16	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1
C 17	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
C 18	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0
C 19	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
C 20	0	0	1	0	0	0	1	0	0	1	0	0	1	1	0	0	1	0	0	0	0
C 21	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
C 22	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1
O 1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
O 3	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
O 4	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
O 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 6	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0
O 7	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 8	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 9	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 10	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0
O 11	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
O 12	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 13	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 14	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 15	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 16	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0

Iso. #	214	217	220	223	230	232	233	234	236	242	245	246	248	251	253	258	260	261	264	265	268
O 17	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0
O 18	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 19	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 20	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
O 21	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 22	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Total	6	1	2	1	1	4	18	11	3	71	1	7	2	1	3	1	22	2	2	4	5

Appendix:		269	270	273	279	285	288	292	296	298	303	305	308	309	312	316	322	329	330	338	343	353
C 1		1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
C 2-1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 2-2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 2-3		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 2-4		1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0
C 2-5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 2-6		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 2-7		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 3-1		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
C 3-2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 3-3		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
C 3-4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 3-5		0	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0
C 3-6		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
C 3-7		0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
C 4-1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
C 4-2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 4-3		0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 4-4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 4-5		0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
C 4-6		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 4-7		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 5-1		0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0
C 5-2		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 5-3		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Iso. #	269	270	273	279	285	288	292	296	298	303	305	308	309	312	316	322	329	330	338	343	353
C 5-4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 5-5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 5-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 6-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 6-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 6-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 6-4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 6-5	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0
C 6-6	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 6-7	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 7	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 8-1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0
C 8-2	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 8-3	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 8-4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
C 8-5	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
C 9	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 10-1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 10-2	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 10-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 10-4	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 11-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 11-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 11-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 12	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 13	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

Iso. #	269	270	273	279	285	288	292	296	298	303	305	308	309	312	316	322	329	330	338	343	353
C 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 15-1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 15-2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
C 16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 17	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
C 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
C 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C 22	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
O 4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
O 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 6	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 16	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0

Iso. #	269	270	273	279	285	288	292	296	298	303	305	308	309	312	316	322	329	330	338	343	353
O 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
O 22	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0
Total	3	2	1	2	2	1	6	1	2	1	5	31	1	2	1	2	1	8	1	6	3

Appendix:		360	379	382	392	393	410	425	430	434	443	459	466	469	483	528	TOTAL
Iso. #		1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	9
C 1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 2-1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 2-2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 2-3		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
C 2-4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
C 2-5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 2-6		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
C 2-7		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 3-1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 3-2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 3-3		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
C 3-4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
C 3-5		0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	12
C 3-6		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
C 3-7		0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	10
C 4-1		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
C 4-2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
C 4-3		1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	7
C 4-4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 4-5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
C 4-6		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
C 4-7		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 5-1		0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	10
C 5-2		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
C 5-3		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6

Iso. #	360	379	382	392	393	410	425	430	434	443	459	466	469	483	528	TOTAL
C 5-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 5-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
C 5-6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 6-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
C 6-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 6-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 6-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 6-5	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	11
C 6-6	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	9
C 6-7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
C 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
C 8-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
C 8-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
C 8-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
C 8-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 8-5	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	7
C 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 10-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
C 10-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	8
C 10-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
C 10-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6
C 11-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 11-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
C 11-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
C 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3

Iso. #	360	379	382	392	393	410	425	430	434	443	459	466	469	483	528	TOTAL
C 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
C 15-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
C 15-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
C 16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
C 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
C 19	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	5
C 20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
C 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
C 22	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	7
O 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
O 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
O 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7
O 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5
O 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
O 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
O 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
O 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
O 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
O 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
O 11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
O 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
O 13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
O 14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
O 15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
O 16	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	8

Iso. #	360	379	382	392	393	410	425	430	434	443	459	466	469	483	528	TOTAL
O 17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
O 18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
O 19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
O 20	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3
O 21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
O 22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Total	2	2	4	1	1	2	1	1	1	1	1	2	1	1	1	

APPENDIX II

Blastn analysis of six isolates of *Daphnia pulex* with different *Pokey* profiles. The sequences shown begin at the 3' end of *Pokey* insertion sites (TTAA) and end at the *Bfa*I site. The length of each of the sequences is in the first bracket, the accession number of the best BLAST match is in the second bracket, and the E value is shown as E. The TE-Display fragments, which begin with the Pok6464F primer and end with the *Bfa*IR linker primer, are 154 nt longer than the sequences shown.

C20

ATAAGGGAGGTATTAGGGGGCAAGATAAGGGACGATGGCGGGAATCATTTTGCGGAAGCC
CCATCTGACCCGGCGGGGAGAATAGGGCTGTCCGTTACATTATTTACCCGCCAAAAACA
TCGAGTGGAGAGCACGGAGAGTAACGATACCACAAACCAGACACATCACACAAGACGATC
ACATTTCCGTAATTTGATTTGTTCTTGTTTGTGTTTTTTCATTAATATTTTCATGCAGCCAC
GCACGTTTTCAAAGACTTATCGTTCCGTTTTCTTTATATCTCAAACCTTTGTTGGCATAT
GGCCATATATGCTTCGATGTCTAAAGGTTAAATCTATTGCCACGAAATCCTTTTCGTGAC
GCGACAGGCTCGACTCACTATTGCTACGAAATCTGAAAATATCGTTTTTCGTAGCAAAAAA
AACC GTT GACTATTGCCACGAAACAAAATAAAGGCAGATAGATGGCGTACAAGAGGCAAA
AGTCAAATTAATAAACGGCAGATAGATGGCGTACAAGTTGCAAAAGTCGTAGCCCGAG
GCGTAGCTAC

(550)

([AC109471.3](#)), *Homo sapiens* chromosome 5 clone RP11-395P13, complete sequence,
E=0.12

C17

TTAACACATCGAAATGAATACACATTCCGTGCAAACCTGTAATGCAGTGTCTTAAATCAA
GATAAAAACTGTCCATGGATTGAAGTCTATAGACGTGTATCTTTTATACCTGAACCGTA
TATGTGAAGTTGAAAATAGAAAGAAATTTCTATTCATTTGCAACGCAAATCTTCTCCAAA
CCCCCATCTTCCAACCTTTGTATATGTACTGTAATTAAGAAGTCGTGTAGTCTCCACCTTC
TTTGTTAAGGGATCGTCCTCGTCCTGCAAGAGCACCTTCTTTTGATTTTCTCATTGACCA
CGTCGAGGATTTGTTGAGAAAAAGTCGTCAGCCCATTTTGAACCGTGGATTTTTTAACG
TTGTCTTACAATGCTGGAATGACCAGATTTTGTGAGCCTTCAAGTTACAACGTTCTAATG
CGATGCGCCATATGTAATGACGTAAACGCCAGTAGAATAAGCTATACGTCGAAGAGTGAT
GAACCAAATGTATTCAGATATGTATCAAGTATAGAGGGACCTAAGCAACGGGTGTTTACT
AC

(542)

([AL935304.8](#)), *Zebrafish* DNA sequence from clone DKEY-29F22 in linkage group 14,
complete sequence, E=0.11

C4-1

TTAAGAGAGTCATAGTTACTCCCGCCGTTTACCCGCGCTTCGTTGAATTTCTTCACTTTG
ACATTTCAGAGCACTGGGCAGAAATCACATTGCGTCAGCACCGACTTGCGGCCATCGCAAT
GCTTTGTTTTAATTAGACAGTCGGATTCCCCCTGGATCGTGCCAGTTCTGAGTTGGTCGTT
CGATGGCGGCCGAGATCTACCACGAGCGCCCCGACTTTTGAGGGCCAAACACTCGGGAGAC
GATGCCGAGCCGCTCCACCGGCAGCGATCTGGCCGAGGACCGAGCCTCAGCGCGAAAGAG
TCCCGAAACGAGAGCTTTCCCCGAAAGAAAAACCAACGCTCGAAACAACCAACGCACTTC
GACCCAGGCCTGACACGTCCGCCGACGGCTTCGCTTGATAAACTCAGCCCGACCGCCTCG
ACCCTCAGAGCCAATCCTTATCCCGAAGTTACGGATCCAATTTGCCGATTTCCCTTACCC
ACATTTCATCCATCGACTAC

(449)

(AF346514.1), *Daphnia pulicaria* large subunit ribosomal RNA gene, partial sequence,
E=0.0

C6-7

TTAATGGATCATCGTGGAACGGTTTCCTTACCACCGAGAAAGTCTCCAAGGATGGCAAAA
TTCCATCAATCACAATCTGAGCCTAAATGATTGTTTAATCAAAGTACCGTGGGAGAAAGG
TCGACCGGGTAAGGGCCCATTTTGGACTCTTGACCCGGACTGTTACGACATGTTTGAGGC
CGGAAACTATACCACCGCCGGAAGCGAAAGCTGAGGATGATCCATCCAGCATCGACTCCC
CCAGCAACGGTGCAACATCATCAGCAGTCTCAACTGAATCGGCGTGAGGCTAAGTTTGTT
TTCAGTATTGAAAATATCCTTCGACCAGATCCGTTACCCGATCAACGGGAGCAGATCACG
CCTCTCCCCTCGTCTCCTGCTATCTACAAAACTTCCTAC

(401)

(AB091061.1), *Dugesia japonica* DjFoxG mRNA for winged helix/forkhead transcription
factor, partial cds, E=0.007

C17

ATAAGCGAACTAAATGTAGTAATTGGTGTTACTTAACAATTAAGTATAGTATCTACTGAA
CAAGTAAGTGGTCATCACTTATAAGTAATTGTTTCCAATTTCGTAACCTTATAAGTGATCCT
AAGTAAATATCCTTGGATTTACAGTGTATTGTGCTAGCGGTTATCGTCGACCGTCCGCTG
TTTAAGGCGGCTCGCTGCGCGAAAAATGTGTAATTGTTGCGCTGTATATAGCGCTATCT
AAGAACGCGTGGCGCTATTATGAACTTAATATTAACCTTGCGTGCAATGCGCTAAACGTAG
CGCAATTAAATTAATTACATAATTTAGAAACGCGCTTCACCTTGCTCAAATAAACTTTTAG
CGCGAAAAATAATGAAATTAATATATTTTTCGCTAC

(396)

(AM483129.2), *Vitis vinifera* contig VV78X082964.7, whole genome shotgun sequence,
E=0.28

C4-1

TTAAGCAATGCCGTCTCCGTTGAATGATTTCGGCCTGTATGCCGACTGGACAGGGACGTAN
AGGTCACCTGGCCANTAAGTGACTTGATANTTGCTTGGCAACCACACGCTCGACCANTTTC
GATAGGAAAGAGAGCANAGATACNGGTCGATAGTTATTANNATCATCCACACAGTGTTGT
GTCTTGGTGCGATTTAAGGATCGCCACTGTATTTAAACGACACACGGGGAATCAACACGA
AAATGTAAATGAGACGGACATGAAAGTAGAACTTGTTCGCATCAAAACGCGAGCCCGAAT
GGCCGCCTCAGCCACCACGCTCGCCGCTCCTCTAC

(335)

(CP000504.1), *Pyrobaculum islandicum* DSM 4184, complete genome, E=0.83

C20

TTAAGACGCCTCGAAACACAGGTAGAAATAGAAAGGCTAAACATAGCACCGCCTTTAATA
CAGAATCATCAGCAAGCCCCGCGGGTACGCATTGATCCCCCGAAATTCTCCGGGGATTGC
CTAAATATTACGGTTGGCGAAACACTTGGAAACACATTCCATGAAAGCCCAAGTTACACA
GACAGTGAAAGGAGCCAGCTATTAGAGGGCCGCACTTCAAGGGGAAGCTAAAACAGCCACA
GATAGATTACGTTCTCGGCAGATACGTACCATAACATCTTACAATTCCTGGCAGACAGA
TTCGGTAACAGAAATACCATAGTCGGCAAACCTAC

(334)

No significant similarity found

C4-1

TTAACCCCCCTAAATGGATAGCTGTAATTAAGCTAAAAATCAGCTTGCCGTTTCGTACGG
GAGAGCACGTTAATTACACACCAGAGCACGCCAGAGCACGCCCGTGACGCCAGAGCACGC
CAGTGACGCCAATTACACACCAGAACACGCCAGAGCACGCCAGTGACGCCAGAGCACGT
CAATGCACGCCAGAGCACGCCAGTGACGCCAGAGCACGCCAGAGCACACCAGTGACACGC
CAGAGCACGCCAATTACACACCAGAGCACGTCAATGCACGCCAGAGCACGCCAGTGACACGC
CAGAGCACGTCAATGCACGCTAC

(323)

(AY619285.1), *Daphnia pulicaria* clone WFms0000276 microsatellite marker Dp267
sequence, E= 3e-32

C5-1

ATAAGGGAGGTATTAGGGGGCAAGATAAGGGACGATGGCGGGGATCATTTTGCGGAAGCC
CCATCTGACCCGGCGGGGAGAATAGGGCTGTCCGTTACATTGCCGGCCCTGTTTTAGTG
GTGAAAACATCTTCTGGATCATCCGCCTCGAGTAAACAAAGTTTGTGTGCTGAATGGATG
TAGCGGGTCGTTTTTCGACTTGATGTCAGAAAGAGTTTTTCGGCCCTTTTGGCAGGTACA
GCGCGAGTGACTTCTACTTCGGCGGACCGGACAGCTCCATCTGCCCTAC

(289)

No significant similarity found

C17

ATAAGGCATAGAAATGTTTTATCTACGAATTTTGACTGGTAACCATCGTGGTTGAAACAA
AATTTCAAGAAATTTATTATTGAAAACAACTATTCATCGCCAACGACATTCCGTATTCC

CAAGCGGTCACCCTTCCAAGTACTAACGGGACTCGACGTAACCTTAACCTTCTGGGAGCTGA
CGAGACCAGGTGCGTTCTACGTGATATGGCCGTTGACATTCAAAAATGAAAATTTACCCT
CCCAGTCCCAATGGATGAACAACATACTAC

(270)

(AJ583108.1), *Venerupis pullastra* 5S rRNA gene, clone TPU2, E=2e-21

C20

TTAGGCAATAAAATGAGACCAACAGTCATAACTGTAAAGTCAAAAAGAAAATTTTGCGGC
AATACCCAATGATGCTTCAACGACCAATCATAACAAAAACACAAATAACTGCCACTAAC
TTTGCCGATGCAAGCGGCCGCGGAGCGGATGCAGAGAGGAGACCTCCAGCGTGTCTCGAG
CCCGCGAGAGGAGTGCTGGAGATTCTCGCGGCTAAAGAACGGAGCCCGCACTGCATAGGT
AACGTTTCCAAGCTAC

(256)

(AC122799.4), *Mus musculus* BAC clone RP23-308K11 from 16, complete sequence,
E=2.1

C5-1

TTAAGTAGTCCATGTGCCTTGTATATTTAGATGATTGTCTTGTTTTCGGTTCCAACCTCG
ACGAATTTTTGGAGCGACTTCATCTCGTTCTAACAACCATTGGTGACGCCGGCCTCACTA
TCAACCCCAAGAAATGTGTGTTTCGCGACGGACAGTGTTTTTCATCTCGGGCACGTCATCG
ACGCCGAAGGTATACGGCCCAATCCGGAAAAAGTAAGTTCCTTTTCTAC

(229)

(AC091910.3), *Homo sapiens* chromosome 5 clone RP11-210I16, complete sequence,
E=6.5

O3

TGAAATGGGAGAGAAAAAAGGCACACCCGGACAACCTCTCCTTTAATCATTCTTTAGTAG
TTGGGACTTATGAAATTGCCGCGCCACGTCTGGTCCCTCTTGGTTACTTAAAGTATCGTT
TGAGGGCTGTTGGTATGTCTTAAATTTTCATGTGTTTGATCATGGACTACTGTAATAAAG
GACGGTACTCTTCGCCTATCTCGACGTGTTAAAGCTAC

(218)

(AC167692.2), *Daphnia pulex* clone JGIAZSN-5P10, complete sequence, E=3e-17

C4-1

ATAAAGGGATAACAGATCTCGATATACGACCGGCAGAGGCAGTGGTCCAGACATTGAAAG
TGAGGCTGAAGGAGTACTCCAAGATGGCCGTTAAATGCCGTAAAACAAGAAAATCAAGAT
AATGCATCGGATGCCAAAAAATGGAGGTTAAGTGTCGTTGGTTTGAACGAGAATTAAAT
CAATCCGAACACCGACTAC

(199)

(BA000032.2), *Vibrio parahaemolyticus* RIMD 2210633 DNA, chromosome 2, complete
sequence, E=5.6

O3

TTATGCAGGGCAAATGCNNACATGCCGCATGTGTGATTCTCCCACCCATGTCTTAGCTAA
TTGCCCCAAGCAAACCCGTAGTTTAACCACTCCCAGCACCATTTCAAAAGGCCCCATTGG
AACACGTAACGTAGTAGTGTACTAACAATCGTGGGCCAGTTAGGACAACTGCTAC

(177)

(AP008208.1), *Oryza sativa* (japonica cultivar-group) genomic DNA, chromosome 2, E= 4.8

C6-7

TTAAGTGCTGAGCTGTTCTCTGCTGGGATCTACCGTGCTCTGGCGTGAACCTGGCATGCAC
TGGCGTGACTGGCGTGCTCTGGCGTGCTCTGGCGAGCTCTGGCGTGCACTGGCGTGCACT
TAGCGTGAACCTGGCGTGCTCTGGCGTGACTAC

(153)

(AY619285.1), *Daphnia pulicaria* clone WFms0000276 microsatellite marker Dp267 sequence, E= 9e-21

C5-1

TTAAGTGCTGAGCTGTTCTCTGCTGGGATCTACCGTGCTCTGGCGTGAACCTGGCATGCAC
TGGCGTGACTGGCGTGCTCTGGCGTGCTCTGGCGAGCTCTGGCGTGCACTGGCGTGCACT
TAGCGTGAACCTGGCGTGCTCTGGCGTGACTAC

(153)

(AY619285.1), *Daphnia pulicaria* clone WFms0000276 microsatellite marker Dp267 sequence, E= 4e-25

C6-7

TTATCATCTCGAAATGATGATACTAATCGTATTTCTTTCTTCGCTGCAAAGCGTGGAAGA
GGTCGTTTAAATGGTAGTGGTACTGGATTAGGAAGATGCAACCCTAC

(107)

(EF514777.1), *Amphidinium carterae* clone shotgun 12mitochondrial sequence, E = 2.5

O3

TTATCATCTCGAAATGATGATACTAATCGTATTTCTTTCTTCGCTGCAAAGCGTGGAAGA
GGTCGTTTAAATGGTAGTGGTACTGGATTAGGAAGATGCAACCCTAC

(107)

(EF514777.1), *Amphidinium carterae* clone shotgun 12mitochondrial sequence, E = 2.5

C20

TTAAGTCAGCAAAACGTCAAATGTTCAATTTTGCAAATAGTTTTTGCATCGAAATTTGTTT
AAAAGGACATTTTTTGGCTTTAAACAAAACCCAGCTCTTCCCTAC

(105)

([DQ786764.1](#)), *Bos taurus* BTA12 scaffold140006_43910 genomic sequence contig
containing highly polymorphic single nucleotide sites, E= 0.20

C4-1

TTATCATCTCGAAATGATACTAATCGTATTTCTTTCTTCGCTGCAAAGCGTGGAAGAGGT
CGTTTAAATGGTAGTGGTATTGGATTAGGAAGATGCAACCCTAC

(104)

([AB228324.1](#)), *Aspergillus oryzae* cDNA, contig sequence: AoEST5197, E=2.4

C20

ATAACAGAAGCGTTGGAGCGCCTTAACACAAAAGTAGAGTCAAATACGTATCAGAACAGA
AGAACAGAGAAGTGTGTTTGGAGCGGGTGGTCTAC

(96)

([CP000679.1](#)), *Caldicellulosiruptor saccharolyticus* DSM 8903, complete genome, E=7.5

C6-7

TTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCACGAATGGATTAACGAGAT
TCCCACTGTCCCTATCTACTATCTAC

(86)

([BA000032.2](#)), *Vibrio parahaemolyticus* RIMD 2210633 DNA, chromosome 2, complete
sequence, E=5.6

C17

TTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCACGAATGGATTAACGAGAT
TCCCACTGTCCCTATCTACTATCTAC

(86)

([AY859574.1](#)), *Eulimnadia texana* internal transcribed spacer 1, partial sequence; 5.8S
rRNA gene and internal transcribed spacer 2, complete sequence; and 28S rRNA gene,
partial sequence, E=3e-35

O3

TTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCACGAATGGATTAACGAGAT
TCCCACTGTCCCTATCTACTATCTAC

(86)

([AY859574.1](#)), *Eulimnadia texana* internal transcribed spacer 1, partial sequence; 5.8S
rRNA gene and internal transcribed spacer 2, complete sequence; and 28S rRNA gene,
partial sequence, E=3e-35

C6-7

TTAAGGTAGCCAAATGCCTCGTCATCTAATTTGTGACGCGCACGAATCGATTAACGAGAT
TCCCACTGTCCCTATCTACTATCTAC

(86)

(AY859574.1), *Eulimnadia texana* internal transcribed spacer 1, partial sequence; 5.8S rRNA gene and internal transcribed spacer 2, complete sequence; and 28S rRNA gene, partial sequence, E=3e-35

O3

TTAANNTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCACGAATGGATTAACGAGAT
TCCCACTGTCCCTATCTACTATCTAC

(86)

(AY859574.1), *Eulimnadia texana* internal transcribed spacer 1, partial sequence; 5.8S rRNA gene and internal transcribed spacer 2, complete sequence; and 28S rRNA gene, partial sequence, E=3e-35

O3

TTAANNTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCACGAATGGATTAACGAGAT
TCCCACTGTCCCTATCTACTATCTAC

(86)

(AY859574.1), *Eulimnadia texana* internal transcribed spacer 1, partial sequence; 5.8S rRNA gene and internal transcribed spacer 2, complete sequence; and 28S rRNA gene, partial sequence, E=3e-35

C4-1

TTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCACGAATGGATTAACGAGAT
TCCCACTGTCCCTATCTACTATCTAC

(86)

(AY859574.1), *Eulimnadia texana* internal transcribed spacer 1, partial sequence; 5.8S rRNA gene and internal transcribed spacer 2, complete sequence; and 28S rRNA gene, partial sequence, E=3e-35

C6-7

TTAAGGTAGCCAAATGCCTCGTCATCTAATTTGTGACGCGCACGAATCGATTAACGAGAT
TCCCACTGTCCCTATCTACTATCTAC

(86)

(AY859574.1), *Eulimnadia texana* internal transcribed spacer 1, partial sequence; 5.8S rRNA gene and internal transcribed spacer 2, complete sequence; and 28S rRNA gene, partial sequence, E=3e-35

C4-1

TTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCACGAATGGATTAACGAGAT
TCCCCTGTCCCTATCTACTATCTAC

(86)

([AY859574.1](#)), *Eulimnadia texana* internal transcribed spacer 1, partial sequence; 5.8S rRNA gene and internal transcribed spacer 2, complete sequence; and 28S rRNA gene, partial sequence, E=3e-35

C6-7

TTACCCAATCGGCATGCCTCTGTGGCGCAATTGGCTAC

(38)

([CR450848.13](#)), *Zebrafish* DNA sequence from clone CH211-149E23 in linkage group 15, complete sequence, E= 1.2

C20

TTGGTTGGCCTAC

(13)

No significant similarity found

O3

TTAANCTAC

(9)

No significant similarity found

O3

TTAANCTAC

(9)

No significant similarity found