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The Molecular Evolution of the Sex-Ratio Gene Complex in *Drosophila persimilis*
(Under the direction of WYATT W. ANDERSON)

The Sex-Ratio X chromosomes of *Drosophila pseudoobscura* and *D. persimilis* are subject to drive, resulting in an excess of female offspring from Sex-Ratio fathers. The several linked loci that cause the Sex-Ratio trait are held together by inversions greatly reducing recombination between the Standard and Sex-Ratio X chromosomes. The *Esterase-5* gene region, tightly linked to the Sex-Ratio complex, was used to infer Sex-Ratio molecular evolution in the sibling species *D. pseudoobscura* and *D. persimilis*. We propose that the Sex-Ratio complex in *D. persimilis* was stabilized when an inversion swept to fixation on the Standard X chromosome. Our data support the hypothesis that the Sex-Ratio chromosome in *D. persimilis* has a monophyletic origin and diverged from the Standard X chromosome approximately 400,000 years ago. This is particularly striking as the Sex-Ratio chromosome of its sibling species, *D. pseudoobscura*, diverged almost a million years earlier.

INDEX WORDS: Meiotic drive, Sex-Ratio, Molecular evolution, Phylogenetic tree, *Esterase-5, D. persimilis*, *D. pseudoobscura*

THE MOLECULAR EVOLUTION OF THE SEX-RATIO GENE COMPLEX IN *DROSOPHILA PERSIMILIS*

by

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Meiotic drive describes a mechanism where one of a pair of heterozygous alleles is transmitted to progeny in excess of the expected Mendelian proportion of 50% (Sandler and Novitski 1957). Expectations would be that preferentially transferred genetic elements would reach fixation, but many examples have been found in which these elements are maintained at low frequencies within populations due to a balancing component of selection. One example of sex-linked drive, known as the Sex-Ratio (SR) trait, has been reported in a number of natural populations of *Drosophila* (Gershenson 1928 and Sturtevant and Dobzhansky 1936). Several forms of autosomal drive have also been reported, such as preferential segregation of a chromosome in maize (Rhoades 1942), *Segregation distorter* (*SD*) in *D. melanogaster* (Novitski 1951), and transmission ratio distortion of the *t*-haplotype in mice (Dunn 1953; and Silver 1982).

In a number of species of *Drosophila*, when a male carries the Sex-Ratio X chromosome (SR X) there is a distortion in the sex-ratio of his progeny (95-100% female). The Sex-Ratio (SR) trait has been observed in a number of natural populations of species from the subgenus *Sophophora*, including *D. affinis* (Morgan et al. 1925), *D. obscura* (Gershenson 1928), *D. pseudoobscura, D. persimilis, D. athabasca, D. azteca* (Sturtevant and Dobzhansky 1936), and *D. subobscura* (Jungen 1967). The trait has also been identified more recently in the subgenus *Drosophila*; including *D. paramelanica* (Stalker 1961), *D. simulans* (Faulhaber 1967), *D. mediopunctata* (De Carvalho et al.

1989), *D. testacea* (James and Jaenike 1990), *D. recens* and *D. quinaria* (Jaenike 1996). In *D. persimilis* and *pseudoobscura*, males carrying the SR X have the capability to produce up to a full set of offspring consisting of 95 to 100 percent daughters. The expression of the SR trait requires the interaction of at least four loci located on the right arm of the X chromosome in *D. persimilis* (Wu and Beckenbach 1983). Many systems of meiotic drive are associated with inversions, which are thought to reduce recombination between the multiple linked alleles causing the trait. The SR X of *D. pseudoobscura* has 3 nonoverlapping inversions on its right arm as compared to the standard X chromosome (ST X). In *D. persimilis*, the SR X has the same gene arrangement as the ST X of *D. pseudoobscura* and differs from the *D. persimilis* ST X by a single, large inversion (Dobzhansky 1939).

In insects, cases of sex-ratio distortion may actually be quite common. If this trend is true, meiotic drive may be a strong force in the evolution of sex-determination mechanisms, sexual selection, and speciation in insects. The SR trait represents an important genetic system: it arose multiple times, in a number of species from different subgroups of *Drosophila*; the gene complex underlying it is tied into a linkage block by various inversions; it has different effects on fertility, virility, and viability; and it may have independently evolved multiple suppressors in the several species. Reconstructing the evolutionary history of the SR drive system and inversion complex will give insight as to how the trait has evolved in different species.

Brady, Richmond, and Oakeshott (1990) cloned and sequenced the *esterase-5* (*Est-5*) gene region enabling us to design primers for amplification and sequencing of the intron region between *Est-5C* and *Est-5B*. The *Est*-5 gene region localized to section 23

within the subbasal inversion of the SR X of *D. pseudoobscura*, but is located between the centromere and the single large inversion of the SR X of *D. persimilis* (Babcock and Anderson 1996). The *Est*-5 gene region proved to be tightly linked to the SR complex of *D. pseudoobscura*, and was very informative to infer a monophyletic origin and estimate the divergence time between the ST X and SR X of *D. pseudoobscura* at 0.7 to 1.3 million years ago (Babcock and Anderson 1996). In an effort to reconstruct the evolutionary history of the SR drive system and inversion complex, we compare additional sequences of the *Est-5* region from ST X and SR X chromosomes in *D. persimilis* and analyze additional nucleotide sequences of ST X and SR X in *D. pseudoobscura* and the X chromosome in *D. miranda*. We present a gene tree, estimated times of divergence, and a proposed model of the evolution of the SR complexes.

CHAPTER 2

THE MOLECULAR EVOLUTION OF THE SEX-RATIO GENE COMPLEX IN *DROSOPHILA PERSIMILIS*[1]

[1]Johnson, E. J., J. Nelson, M. A. F. Noor, W. W. Anderson. To be submitted to Molecular Biology and Evolution.

Abstract

The Sex-Ratio X chromosomes of *Drosophila pseudoobscura* and *D. persimilis* are both subject to drive, which results in an excess of female offspring from Sex-Ratio fathers. The several linked loci that cause the Sex-Ratio trait are held together by inversions on the right arm of the X chromosome that greatly reduce recombination between the Standard and Sex-Ratio X chromosomes. The *Esterase-5* gene region, tightly linked to the Sex-Ratio gene complex, was used to infer Sex-Ratio molecular evolution in the sibling species *D. pseudoobscura* and *D. persimilis*. Sequences were compared from 14 Sex-Ratio and 14 Standard X chromosomes from *D. persimilis*, 21 Sex-Ratio and 22 Standard X chromosomes from *D. pseudoobscura*, and 4 X chromosomes from *D. miranda*, supplemented with sequences from an earlier study. The addition of data from *D. persimilis* revealed an intriguing phylogeny for the Sex-Ratio chromosomes. We propose that the Sex-Ratio complex in *D. persimilis* was stabilized when an inversion dramatically increased in frequency on the Standard X chromosome. Our data support the hypothesis that the Sex-Ratio chromosome in *D. persimilis* has a monophyletic origin and diverged from the Standard X chromosome approximately 400,000 years ago (about 1.2 million generations). This is particularly striking as the Sex-Ratio chromosome of its sibling species, *D. pseudoobscura*, diverged almost a million years earlier.

Introduction

Meiotic drive describes a mechanism by which one of a pair of heterozygous alleles or heteromorphic chromosomes is transmitted to progeny in excess of the expected proportion of 50% (Sandler and Novitski 1957). Many traits originally considered to be meiotic drive do not involve meiosis directly, and the term *drive* is now used to describe the general phenomenon of deviation from Mendelian proportions among offspring. For example, one type of sex-linked drive of the X chromosome against the Y chromosome is known as the Sex-Ratio (SR) trait. SR fathers produce almost all X-bearing sperm, therefore 95-100% of the progeny are female. The SR trait has been reported in a number of natural populations of *Drosophila*, in 7 species from the subgenus *Sophophora* and more recently in 6 species from the subgenus *Drosophila*. The SR trait in *D. pseudoobscura* has been found at frequencies of up to 33% within populations from western North America (Wallace 1948). The SR X chromosome in *D. pseudoobscura* (SR^{psd}) exhibits a clinal increase in frequency with decreasing latitude and altitude, reaching a maximum near the Mexican border (Sturtevant and Dobzhansky 1936; Dobzhansky 1944; and Anderson, Dobzhansky, and Kastritsis 1967). The SR X chromosome of *D. persimilis* (SR^{per}) ranges along the coast of California and Oregon (Sturtevant and Dobzhansky 1936; and Dobzhansky 1944).

Genetic elements subject to drive might be expected to reach fixation if there is no balancing component of selection associated with the element, such as decreased viablility, virility, or fertility. The SR X chromosomes are often found to be maintained at low to moderate frequencies. The SR trait in *D. simulans* appears to cause abnormal segregation of the Y chromosome during meiosis, and the resulting abnormal spermatids

are then eliminated after elongation (Cazemajor, Joly, and Montchamp-Moreau 2000). A similar mechanism may operate in *D. persimilis* and *D. pseudoobscura* where males carrying the SR X have the capability to produce offspring consisting of 95 to 100 percent daughters. Policansky and Ellison (1970) discovered these males have a conditional fertility deficiency because a SR male produces only half as many sperm as a standard (ST) male per unit time. Males carrying the SR X suffer a substantial virility reduction after repeated matings or when mated to non-virgin females (Beckenbach 1978; and Wu 1983). Furthermore, as the frequency of the SR X chromosome increases in a population, so does the proportion of females. In turn, the potential rate of male mating increases, and SR males sould be expected to exhaust their sperm supply more rapidly, with the result that ST males would contribute more offspring to the next generation (Capillon and Atlan 1999). Thus, a frequency-dependent selection is believed to keep the SR frequency in check.

The expression of the SR trait requires the interaction of at least four alleles at loci located on the right arm of the SR^{per} (Wu and Beckenbach 1983). Many systems of meiotic drive have inversions, which reduce recombination between the linked genes associated with the trait (Lyttle 1991). Both *D. persimilis* and *D. pseudoobscura* have inversion differences between their ST and SR X chromosomes (Sturtevant and Dobzhansky 1936). These inversions are not thought to be responsible for the SR trait but are believed to have bound the SR alleles together by reducing recombination, allowing for continued transmission of the SR trait (Wu and Beckenbach 1983). The SR^{psd} has 3 nonoverlapping paracentric inversions on its right arm in comparison to the ST X chromosome (ST^{psd}) (Sturtevant and Dobzhansky 1936; and Dobzhansky 1939).

The SR^{per} has the same gene arrangement as the ST^{psd} and differs from the *D. persimilis* ST X (ST^{per}) by the single, large inversion (Dobzhansky 1939). The inversions differing between the ST^{psd} and SR^{psd} reduce recombination between the *forked* and *magenta* mutations from 20% to 4.5%, and between *magenta* and *short* from 47% to 0.5%. In *D. persimilis*, recombination between the *forked* and *short* mutations is reduced from 47.1% to 4.4% (Sturtevant and Dobzhansky 1936; Beckenbach 1981). Recombination is suppressed similarly in both species, and the *Esterase-5* (*Est-5)* gene region is tightly linked to the SR trait in both species (Beckenbach, Curtsinger, and Policansky 1982).

Babcock and Anderson (1996) studied the evolutionary history of the SR drive system and inversion complex in *D. pseudoobscura*, using the cloned and sequenced *Est-5* locus in *D. pseudoobscura* (Brady, Richmond, and Oakeshott 1990). The *Est*-5 gene region is localized to section 23 within the subbasal inversion of the SR^{psd}, but is located between the centromere and the single large inversion of the ST^{per} (Babcock and Anderson 1996). In a molecular analysis of several X linked loci, Kovacevic and Schaeffer (2000) failed to reject a selectively neutral hypothesis for the *Est-5* locus using the Tajima (1989), Fu and Li (1993), and Hudson-Kritman-Aguade (Hudson, Kreitman, and Aguade 1987) tests, and they found that the locus was very tightly linked to the SR trait. Babcock and Anderson (1996) used the intron between *Est*-5C and *Est*-5B in *D. pseudoobscura* to infer a monophyletic origin of the SR^{psd}, and to estimate the divergence time between ST X and SR^{psd} at 0.7 to 1.3 million years ago. This divergence predates the split between *D. pseudoobscura* and *D. persimilis*. Unfortunately, only one strain of *D. persimilis* carrying the SR^{per} was available to Babcock and Anderson for comparison

with the SR^{psd}, and it was not possible to accurately infer the evolutionary relationship of SR in the two species.

The SR trait represents an important genetic system: it arose multiple times, in a number of species from different subgroups of *Drosophila*; the gene complex underlying it is tied into a linkage block by various inversions; it has different effects on fertility, virility, and viability; and it may have independently evolved multiple suppressors in the several species. In an effort to reconstruct the evolutionary history of the SR drive system and inversion complex, we compare additional sequences of the *Est-5* region from ST^{per} and SR^{per} chromosomes in *D. persimilis* and analyze additional nucleotide sequences of ST^{psd} and SR^{psd} in *D. pseudoobscura* and the X chromosome in *D. miranda*. We present a gene tree, estimated times of divergence, and a proposed model of the evolution of the SR complexes.

Materials and Methods

Fly Strains

We compared seventy-one *D. pseudoobscura* strains, 36 carrying the ST^{psd} and 35 carrying the SR^{psd}. Twenty-two *D. pseudoobscura* strains carrying the ST^{psd} were used from stocks that had been collected and maintained from sites in Arizona, California, Colorado, New Mexico, Nevada, Texas, and Utah (Table 1). Twenty-one *D. pseudoobscura* strains carrying the SR^{psd} were used from stocks from the same collection sites. In addition, 28 *D. pseudoobscura* sequences from Babcock and Anderson (1996) were included in the analysis, 14 of which carried the SR^{psd}, and 14 the ST^{psd}.

Previously, the absence of a visible marker on the XR in *D. persimilis* prevented the isolation and maintenance of *D. persimilis* SR strains. To provide an XR-linked marker in *D. persimilis*, the *short* mutation from *D. pseudoobscura* was introgressed into *D. persimilis*, with repeated backcrossing for two years to restore the *D. persimilis* background. This mutation enabled us to isolate SR stocks of *D. persimilis* and maintain them with the crossing scheme shown in Babcock and Anderson (1996). We included thirty-four *D. persimilis* strains from recent collections, 14 carrying the ST^{per} from sites in California and Texas, and 14 carrying the SR^{per} from California. Five *D. persimilis* strains carrying the ST^{per} chromosome from California and one strain from British Columbia, which had been sequenced by Babcock and Anderson (1996), were used to supplement the analysis. A total of 7 strains of *D. miranda*, another close relative, were used to aid in rooting of the phylogenetic trees. Four stocks maintained from collection sites in California and Oregon were used, along with 3 strains from Babcock and Anderson (1996).

DNA Extraction

Genomic DNA was extracted from single male flies using the protocol of Gloor and Engels (1992) except incubation was performed at 55°C instead of 37°C, using a PTC-100 thermal cycler from MJ Research, Inc.

PCR Amplification and Sequencing

The *Est-5* gene consists of 3 tandem copies, designated *Est-5A*, *Est-5B*, and *Est-5C*. Primers were designed from the published sequence of the *D. pseudoobscura* gene

region (Brady, Richmond, and Oakshott 1990). These primers (DE5C'S1: 5' CGATAAGTCGAGCCTCTCTCTATG 3' and DE5B5'N1: 5' AACCAGTCTCAGGGGGATAGCTCT 3'), used in Babcock and Anderson (1996), amplify a 587 basepair region of noncoding DNA from the intron between the *Est-5B* and *Est-5C* open reading frames. PCR amplification included 10µl of extracted DNA as template, 10pm of each primer, and either Promega Taq polymerase (.5µl) or Qiagen Taq PCR Master Mix, for a total volume of 50µl. Thermal cycling was performed in a Stratagene Robocycler as described by Babcock and Anderson (1996). PCR products were sequenced in both directions by sequencing facilities at the University of Georgia or the Louisiana State University Museum of Natural Science.

Sequence Analysis

The program Sequencher 3.0 (Gene Codes Corporation) was used to align sequences and discern ambiguities, and alignment adjustments were then made. Sequences were trimmed to include 512 total nucleotides, of which 435 were invariant. Average heterozygosity per nucleotide site within and between populations were calculated using the Molecular Evolutionary Genetics Analysis (MEGA) package (Kumar, Tamura, and Nei 1994).

Estimated times of divergence were calculated by using the average heterozygosity between populations. We used a substitution rate of 1.7% sequence divergence per million years along two lineages (Caccone, Amato, and Powell 1988). Three generations per year was used for estimating the number of generations since divergence.

Phylogenetic analysis was performed using the method of neighbor joining with distances corrected by the Jukes-Cantor relationship, and the methods of maximum likelihood and parsimony with branch and bound searches. Each of these was performed using PAUP 4.0b3 (Swofford 1993), PHYLIP version 3.573c (Flesenstein 1993), or MEGA (Kumar, Tamura, and Nei 1994), with the same conclusions drawn from the comparable trees for the three analysis packages.

Results and Discussion

Nucleotide Diversity

At site 55, all 15 *D. persimilis* strains with the SR^{per} carry a C instead of a T. This difference supports the assumption that no recombination occurred between the end of the inversion and the *Est-5* gene and that *Est-5* is tightly linked to the SR gene complex. At site 12, most of the strains from this group carry C instead of an A and have a deletion at site 15. Furthermore, the polymorphism within SR^{per} is very low, with 10 of 15 strains identical. The average heterozygosity per nucleotide was 0.10% with a S.E. of 0.08%. This figure may be an overestimate because one strain carried an 11 base pair deletion. The SR^{per} possesses almost 4-fold less diversity than the ST^{per}. Since the SR^{per} is maintained at a low frequency (0-25%) within populations, it has a smaller effective population size than the ST^{per}. The expected value of heterozygosity is directly proportional to the population size (Kimura 1968), likely accounting for the reduced variation.

There is not a single nucleotide from the ST X chromosomes that is unique to either *D. pseudoobscura* or *D. persimilis*, suggesting extensive shared ancestral

polymorphism or gene flow. At sites 256, 269, 280, twenty ST^{psd} and one ST^{per} strains have all three mutations, A→C, A→C, and T→C, respectively. Another 11 ST^{psd} and 3 from ST^{per} strains carry at least one of these 3 differences. The remaining 5 ST^{psd} and 14 ST^{per} have the original A, A, T configuration as do strains from both species when they are carrying SR X. ST^{psd} strains share more ancestral nucleotides than *D. persimilis*. At site 16, 17 of the 36 strains of ST^{psd} carry an ancestral C in common with all the *D. miranda* strains. Five of the ST^{psd} strains carry A at site 117, as do all the SR^{psd} strains. In addition, at site 202, 7 of the 36 ST^{psd} strains carry a C, as do all the *D. miranda* strains.

The average heterozygosity of the ST^{psd} is 1.32% with a S.E. of 0.25%, while the ST^{per} is only 0.39% with a S.E. of 0.11%. Heterozygosity within the ST^{per} is thus about 3.5 fold less than in the ST^{psd}. Although effective population size of *D. pseudoobscura* is larger than *D. persimilis*, the average heterozygosity at other loci are very similar within ST^{psd} and ST^{per}. The *Alchohol dehydrogenase* region on the fourth chromosome is just 1.12 fold higher in *D. perimilis*, and the *Period* locus on the XL is 1.2 fold higher in *D. pseudoobscura*. *Heat-shock protein 82* is localized to the same region of the XR as *Est-5* and also has an average heterozygosity that is 3.5 fold less in *D. persimilis* than *D. pseudoobscura* (Wang, Wakeley and Hey 1997). Since the two species have an inversion difference between their ST X chromosomes, a dramatic increase in frequency of this inversion on the ST^{per} would explain the reduced variation found on the ST^{per}. In addition, the fact that the *Est-5* locus in *D. pseudoobscura* is one of the most polymorphic enzymes found in Drosophila, with 41 different alleles identified (Keith 1983), argues that there must have been an enormous amount of polymorphism maintained in the

ancestral species. The ST^{psd} has the same chromosomal arrangement as SR^{per}, but Wu and Beckenbach (1983) found extensive differentiation between them, so it is improbable that the recent inversion could have occurred prior to their divergence. Therefore, it appears the inversion occurred on the ST^{per}.

The X chromosome of *D. miranda* and SR^{psd} are highly diverged from the rest of the arrangements. Both groups have sequence identity at some of the variable sites, notably at sites 191 (T), 231 (T), and 241 (A). *D. miranda* diverged approximately 2 million years ago from *D. pseudoobscura* and *D. persimilis* (Aquadro et al. 1991) and is used as the outgroup in this study. There are eight nucleotide sites (33, 88, 144, 251, 255, 317, 336, 406, and 407) at which all the *D. miranda* strains differ from the other two species (see Table 2). The SR^{psd} strains have also had enough time since their divergence to have two mutations and a deletion arise that only occur within this chromosome (sites 39, 172-176, and 239). The hterozygosity within *D. miranda*, 0.29%, and SR^{psd}, 0.13%, is very low. The average heterozygosity within the SR^{psd} was about 10-fold less than that for the ST^{psd}, which is consistent with the findings of Babcock and Anderson (1996). The low observed variation for SR^{psd} probably results from a combination of the low chromosome frequency maintained in the species, a monophyletic origin, and the accumulation of the 3 non-overlapping inversions all of the SR^{psd} carry. The low average heterozygosity in *D. miranda* is probably evidence of a small population size.

There was no geographical pattern associated with sequence identity within any of the populations. Identical sequences of each population were from random locations with no apparent relation to distance. For example, the 10 identical sequences from the ST^{per} were from locations ranging from Death Valley to James Reserve, Mather, and

Mount St. Helena in California, and to Spray in Oregon, while other strains from Mather and Mount St. Helena closely resembled sequences from the ST^{psd}. Furthermore, some of the SR^{per} collected from Mount St. Helena were identical to those from Mather. One strain differed by one nucleotide from a strain collected in British Columbia, and one strain had a unique 11 bp deletion. These observations are consistent with the high levels of intraspecific gene flow documented for these species (e.g., Prakash et al. 1969; Schaeffer and Miller 1992; Noor et al. 2000).

Estimated Divergence Times

Kovacevic and Schaeffer (2000) recently showed that the *Est-5* marker in *D. pseudoobscura* should give an unbiased estimate of gene flow because it failed to reject a neutral mutation hypothesis when the statistical tests of Tajima (1989), Fu and Li (1993), and Hudson-Kritman-Aguade (Hudson, Kreitman, and Aguade 1987) were applied. Although the noncoding region that was sequenced is within the intron between the *Est-5C* and *Est-5B* genes, it may still contain regulatory information that would cause some selective constraints (Brady et al 1990). Goddard, Caccone, and Powell (1990) used DNA-DNA hybridization to study DNA divergence in the *obscura* group. They found that although chromosomal evolution, in terms of the number of inversions, has been much more rapid than in the *melanogaster* subgroup, evolution at the nuclear level is about the same and may be even slower. Therefore we used a substitution rate of 1.7% sequence divergence per million years along two lineages, as determined from DNA-DNA hybridization of single copy nuclear DNA between species in the *melanogaster* subgroup (Caccone, Amato, and Powell 1988).

Our estimates of divergence are congruent with previous estimates by Babcock and Anderson (1996) and Aquadro et al. (1991). *D. pseudoobscura* and *D. miranda* shared a common ancestry over 2 mya or 6 million generations ago. We estimate the split of *D. pseudoobscura* and *D. persimilis* to be approximately 700,000 years ago. Our estimate for divergence time between the SR^{psd} and ST^{psd} is almost 1.4 mya, slightly greater than estimated by Babcock and Anderson (1996). Finally, our estimate for the divergence time between the SR^{per} and ST^{per} is just over 300,000 years ago, or about 1 million generations. It is interesting that the two SR X chromosomes have not shared a common ancestor for more than half a million years, thereby discounting the possibility of an introgression event from one species to the other, like the introgression for the *t*-complex in mice proposed by Wu and Hammer (1991).

Phylogenetic Analysis

We used 3 methods of phylogenetic analysis. We present the neighbor joining tree produced by MEGA (Fig. 2), since the three methods were comparable. A gene tree allows us to examine linked regions of a genome under increased evolutionary pressure while assuming the rest of the genome evolved in accordance with its species. After *D. miranda*, the outgroup, branched off from the ancestral species, the SR^{psd} was the next to diverge, with a very deep branch and well-supported clade (99% of 500 replicates). This node was also fully supported in the maximum likelihood and parsimony trees we generated (data not shown). The evidence that SR^{psd} diverged *before* the species split with *D. persimilis* is well supported by our data and in agreement with previous

conclusions (Babcock and Anderson 1996). After the species split, the SR^{psd} was maintained in *D. pseudoobscura* but eliminated from *D. persimilis*.

The additional data for the ST^{psd} and ST^{per} make it difficult to clearly separate the species divergence at any particular clade. To distinguish the split of the two species and the SR^{per}, more sequence data is needed for bootstrap support, because of their recent divergence. The ST X chromosomes in the two species cannot be separated because there is not one single nucleotide that differs between them. The SR^{per} forms a cluster, with bootstrap support of 58% of 500 replicates, that branchs off from ST^{per}. In the SR^{per}, one nucleotide that all the strains share has changed, as have two nucleotides that most strains share since its divergence from ST^{per}. The SR^{per} appears to have a monophyletic origin, which is closely related to ST^{per}.

Model of Evolution

The data support an intriguing model of evolution for the SR gene complexes in *D. pseudoobscura* and *D. persimilis*. We found that the two SR gene complexes, associated with inversions, each have a monophyletic origin and that they arose almost 1 million years apart. At least 4 loci and possibly more must all have SR alleles present at the same time for the SR trait to be expressed in *D. persimilis*. Wu and Beckenbach (1983) proposed that many of the SR alleles were ancestral and that the ST alleles were derived later because the carriers of SR were less fit than carriers of ST, especially SR/SR females have significantly reduced viability and fecundity (Wallace 1948; and Beckenbach 1983). Our data support this hypothesis, and one can imagine that SR alleles were present in the ancestral population but were seldom all expressed together. We

found ST^{psd} to have much more variation than any of the other X chromosome types we tested, and the ancestral species most likely maintained this extensive variation with various combinations of the ST and SR alleles on each chromosome. Rare events would still occur in which recombination would bring all the SR alleles together on the uninverted chromosome and the SR trait would be expressed, but recombination would soon separate them. Only when one or several inversions reduced recombination would all the SR alleles be held together.

In the ancestral species, inversions arose on the SR^{psd} prior to the species split. This SR^{psd} was only maintained in *D. pseudoobscura*. The ST^{psd} may still have SR alleles which are rarely expressed together as the SR trait, but again recombination would soon separate the alleles and prevent their expression as the SR trait. After the species split, an inversion arose on the ST^{per}, which expressed a ST phenotype. SR carrriers were less fit than ST carriers (Beckenbach 1983), and when this inversion arose reducing recombination, the ST alleles were locked together, causing this chromosome to have a higher fitness. Its frequency increased in *D. persimilis*, explaining the ST^{per} arrangement. The ancestral ST X chromosome was eliminated because of competition with the new inverted ST^{per} presumably carrying a fitness-enhancing complex of genes. A chromosome carrying all the SR alleles together was maintained in *D. persimilis*, because reduced recombination with the inverted ST^{per} would hold the SR alleles together, and the drive would allow it to compete successfully with the inverted ST^{per}.

The analysis of other gene regions linked to the SR complex may help to strengthen support for our model of the evolution of SR in these two species. Many

questions remain, such as how old are the SR alleles and whether they play a part in the evolution of the SR trait in other species of *Drosophila*.

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CHAPER 3

CONCLUSIONS

In our study, the evolution of the Sex-Ratio drive system in *D. persimilis* was based upon a comparison of the nucleotide sequences from the *esterase-5* gene region, which is tightly linked to the SR inversion complex. Our data gave additional support to the model of evolution for the SR complex in *D. pseudoobscura* from Babcock and Anderson (1996). The SR X chromosome of *D. pseudoobscura* appears to have a monophyletic origin in *D. pseudoobscura* and a divergence time of approximately 1.3 million years ago prior to the species split. The multiple SR alleles on the right arm of the X chromosome appear to be held together by 3 separate inversions.

The evolution of the SR complex in *D. persimilis* suggests that the SR alleles and ST alleles were maintained in various combinations on the ST X chromosome in the ancestral species. The expression of the trait would only occur in rare events when the multiple SR alleles were together on one chromosome, although recombination would prevent this chromosome from reaching a stable frequency. After the species split, an inversion occurred on the right arm of the X chromosome of *D. persimilis*, which carried at least one of the ST alleles. Since ST carriers are more fit than SR carriers (Beckenbach 1983), this inverted ST chromosome probably increased in fitness. Selection for the inverted chromosome caused it to increase towards fixation. The inversion was sufficient to suppress recombination with an univerted chromosome carrying the multiple SR alleles, effectively keeping the SR alleles locked together and allowing for expression

of the SR drive system. Therefore, the SR complex of *D. persimilis* was stabilized when the inversion increased in frequency on the ST X chromosome approximately 300,000 years ago after the species split.

The SR gene complexes were stabilized by inversions almost 1 million years apart, and both SR chromosomes had monophyletic origins.

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APPENDIX A

TABLES

Table 1. Drosophila X chromosome terminology and site of origin

Strain Origin	Standard X	Sex-Ratio X
D. pseudoobscura		
American Fork Canyon, UT		SRPSDAFC1
		SRPSDAFC11
Albuquerque, NM	XAL	SRAL
Black Canyon, CO	XBC	SRBC
Bryce Canyon, UT	PSDBRY127	SRPSDBRY9
	PSDBRY150	SRPSDBRY10
Chiracahua, AZ	PSDCH252	SRPSDCH247
	PSDCH349	
Charleston Mountains, NV	PSDCM20	SRPSDCM7
	PSDCM121	SRPSDCM24
Davis Mountains, TX	PSDDM28	SRPSDDM997
	PSDDM54	SRPSDDM1087.
Flagstaff, AZ	PSDFL9	SRPSDFL7
	PSDFL13	SRPSDFL125
	PSDFL14	
	PSDFL16	
Grand Canyon, AZ	XGC	SRGC
		SRPSDGC627
		SRPSDGC801
James Reserve, CA	PSDJR274	SRPSDJR38
	PSDJR292	SRPSDJR174
Mount St. Helena, CA	PSDMSH9	SRPSDMSH324
	PSDMSH24	
Mesa Verde, CO	PSDMV67	SRPSDMV3
	PSDMV535	SRPSDMV69
North Rim, AZ	PSDNR7	SRPSDNR2
	PSDNR109	SRPSDNR8
Ruidoso, NM	PSDRUI17	SRPSDRUI1
	PSDRUI152	
San Bernardino, CA	XSB3	SRSB3
	XSB4	SRSB4
	XSB6	SRSB6
	XSB7	SRSB7
	XSB9	SRSB9
	XSB10	SRSB10
	XSB13	SRSB13
	XSB16	SRSB16
	XSB18	SRSB18
Sierra Mountains, CA	XSM	SRSM
Tempe, AZ	XTE	SRTE

Table 1 continued

Strain Origin	Standard X	Sex-Ratio X
D. persimilis		
British Columbia		SRPER
Davis Mountain, TX	PERDM18	
Death Valley, CA	PERDV2.2	
	PERDV118	
	PERDV183	
	PERDV223	
	PERDV317	
	PERDV321	
James Reserve, CA	PER	
	PERJR105	
Mather, CA	PER1	SRPERMA44
	PER2	SRPERMA49
	PER75	
	PERMA148	
	PERMA150	
	PERMA171	
Mount St. Helena, CA	PERMSH3	SRPERMSH3
	PERMSH4	SRPERMSH7
	PERMSH5	SRPERMSH34
		SRPERMSH39
		SRPERMSH43
		SRPERMSH82
		SRPERMSH160
		SRPERMSH226
		SRPERMSH231
		SRPERMSH294
		SRPERMSH421
		SRPERMSH1998
Spray, OR	PERSP101	
D. miranda		
Mather, CA	MIRMA23	
	MIRMA28	
	MIRMA83	
Mount St. Helena, CA	MIRMSH38	
Drosophila Center	MIRDC	
Spray, OR	MIRSP138	
	MIRSP23	

Table 2. Between-group polymorphisms

Nucleotide #	4	1	1	1	2	3	5	8	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2	2	3	3	4	4	4
		2	5	6	3	9	5	8	1	4	7	7	7	7	7	9	0	3	3	4	5	5	5	6	8	8	1	3	0	0	2
									7	4	2	3	4	5	6	1	2	1	9	1	1	5	6	9	0	2	7	6	6	7	8
consensus	G	A	T	G	A	G	T	T	G	C	G	T	A	C	T	C	A	G	C	G	A	G	A	A	T	C	T	G	C	A	C
D. miranda	T	.	.	C	G	.	.	C	.	G	.	.	.	.	.	T	C	T	.	A	T	-	C	.	.	.	A	C	T	T	T
SR *D. pseudoobscura*	.	.	.	.	.	A	.	.	A	.	-	-	-	-	-	T	.	T	A	A	.	.	.	.	.	A/.	.	.	.	.	.
ST D. pseudoobscura	.	.	.	C/.	.	.	.	.	-/A	.	.	.	.	.	.	.	-/C	.	.	.	.	.	C/.	C/.	C/.	.	.	.	.	.	.
ST D. persimilis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-/C	-/C	-/C	.	.	.	.	.	.
SR *D. persimilis*	.	C/.	-/.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

* shared nucleotide by majority of group/shared nucleotide by minority of group

Table 3. Within-group polymorphisms

A. *D. miranda*

Nucleotide #	4	8	1	1	2
		5	2	8	9
			1	3	2
consensus	T	C	A	A	T
Miranda(5)	.	.	.	.	.
MIRMA23	G	T	.	.	A
MRSP138	.	.	C	G	.

B. SR *D. persimilis*

	1	1	3
	2	5	9
consensus	C	-	G
SRPER(10)	.	.	.
SRPERMSH(3)	A	T	.
SRPER	A	T	T
SRPERMSH43	*	.	.

*deletion from 2-11

C. SR *D. pseudoobscura*

Nucleotide #	1	2	3
	9	8	6
	0	2	1
consensus	A	A	T
SRPSD(15)	.	.	A
SRPSD(8)	.	C	.
SRPSD(9)*	.	C	.
SRPSDAFC11	.	.	.
SRSM	T	.	.

*insertion from 412-415

D. ST *D. persimilis*

	1	3	4	1	1	2	2	2	2	2	2	2	3	4	4
	0	4	4	1	9	1	1	1	5	5	6	8	2	0	0
				0	0	2	4	7	6	9	9	0	5	3	4
consensus	T	G	C	G	A	G	G	A	A	G	A	T	A	C	C
PER(14)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PERDM18	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
PERDV118~	.	.	.	.	.	C	.	G	.	.	.	.	.	A	T
PERDV183	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PERDV317	C	.	.	.	.	.	.	.	C	.	.	.	C	.	.
PERDV321	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
PERMA150	.	.	.	C	.	.	.	.	C	.	C	C	.	.	.
PERMA171	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.
PERMSH4	.	A	-	.	.	.	.	.	*	*	C	C	.	.	.
PERMA2	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.

* deletion from 234-263

~insertion from 395-402

Table 3 continued

E. *ST D. pseudoobscura*

Nucleotide #	6	9	1	3	4	4	9	1	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4
			6	2	4	7	8	1	5	5	6	9	0	0	1	1	1	3	4	4	4	5	5	6	7	7	8	0	0	0	0	0	0	3	6	7	9	0	0	1	1	2	2	3	3	4	9
								7	8	9	2	0	2	5	1	6	8	0	3	6	9	6	8	9	2	3	0	4	5	6	7	8	9	6	5	7	1	5	8	0	9	6	8	3	4	4
consensus	G	A	C	A	C	A	A	G	C	A	G	A	A	T	C	G	G	G	C	G	A	C	C	C	C	T	C	A	T	A	C	C	C	G	G	A	T	A	T	T	G	T	C	A	C	A
PSD(2)	.	.	.	.	.	G	.	.	.	T	.	.	.	.	.	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDBRY150	.	.	G	.	.	.	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	C	.	.	.	.
PSDCH252	.	.	.	.	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDCH2349	.	.	.	.	.	G	C	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDCM121~	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	T	G	G	G	T	A	T	.	A	.	.	.	C	.	.	.	.	.	.	.
PSDCM20	.	.	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
PSDDM28	.	.	G	.	.	.	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
PSDDM54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDFL13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDFL14	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDFL16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	A	.	.	T	.	.	.	.	.	.	A	.	G	.	.	.	.	.	.	.	.	.	.
PSDFL9	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDJR274	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDJR292	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
PSDMSH9	.	.	G	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDMV535	.	C	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDMV67	.	.	.	.	.	G	.	.	T	.	.	.	.	.	.	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PSDNR109	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.
PSDNR7	.	.	G	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
PSDRM(2)	.	.	G	.	.	.	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
PSDRUI17	.	.	.	.	.	.	.	A	.	.	.	.	C	.	.	.	.	.	.	.	.	A	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
XAL	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
XBC	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	T	G	.
XGC	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
XSB10	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
XSB13	A	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
XSB16	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	A	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
XSB18	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
XSB3	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	G	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	A	.
XSB4	.	.	.	.	.	G	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	A	*	*	*	*	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
XSB(2)	.	.	G	.	.	.	.	.	T	.	.	.	C	T	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
XSB7	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.
XTE	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	A	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

* deletion from 256-275

~insertion from 412-415

Table 4. Within-group heterozygosity

	H	S.E.
D. miranda	0.00286	[0.00122]
SR *D. pseudoobscura*	0.00127	[0.00098]
ST *D. pseudoobscura*	0.01324	[0.00256]
ST *D. persimilis*	0.00386	[0.00111]
SR *D. persimilis*	0.00103	[0.00076]

H = average heterozygosity per nucleotide
S.E.= standard error

Table 5. Between-group heterozygosity

	1	2	3	4	5
1. *D. miranda*		[0.00745]	[0.00716]	[0.00718]	[0.00741]
2. SR *D. pseudoobscura*	0.03436		[0.00552]	[0.00491]	[0.00547]
3. ST *D. pseudoobscura*	0.03754	0.02325		[0.00259]	[0.00338]
4. ST *D. persimilis*	0.03409	0.01508	0.01137		[0.00233]
5. SR *D. persimilis*	0.03639	0.01663	0.01401	0.0055	

bottom left = average heterozygosity per nucleotide
top right [ ] = standard error

Table 6. Divergence Estimates

	1	2	3	4	5
1. *D. miranda*		[0.43824]	[0.42118]	[0.42235]	[0.43588]
2. SR *D. pseudoobscura*	2.02118		[0.32471]	[0.28882]	[0.32176]
3. ST *D. pseudoobscura*	2.20824	1.36765		[0.15235]	[0.19882]
4. ST *D. persimilis*	2.00529	0.88706	0.66882		[0.13706]
5. SR *D. persimilis*	2.14059	0.97824	0.82412	0.32353	

(substitution rate of 1.7% sequence divergence per million years)
bottom left = estimated time of divergence (millions of years ago)
top right [ ] = standard error (millions of years ago)

APPENDIX B

FIGURES

Figure 1. Representation of inversions [ ] and the *Est-5* gene region as determined from salivary chromosomes and in situ hybridization, and positions of *f*, *mg*, and *s* based on genetic maps.

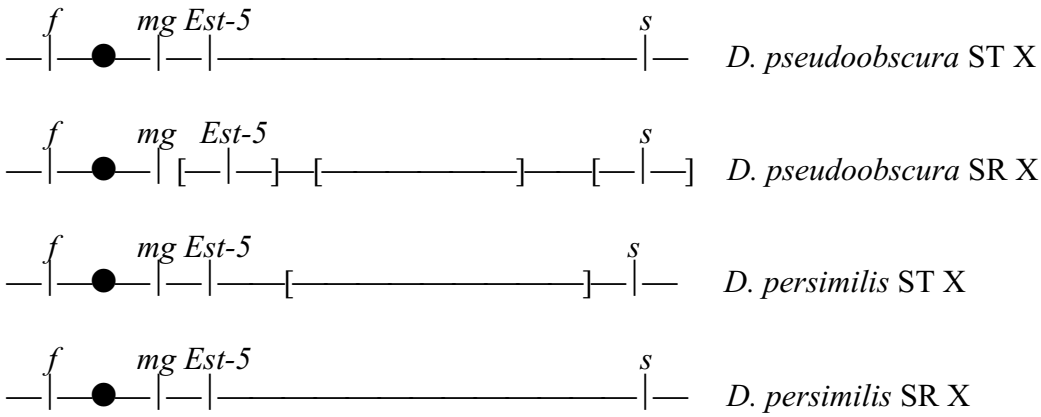
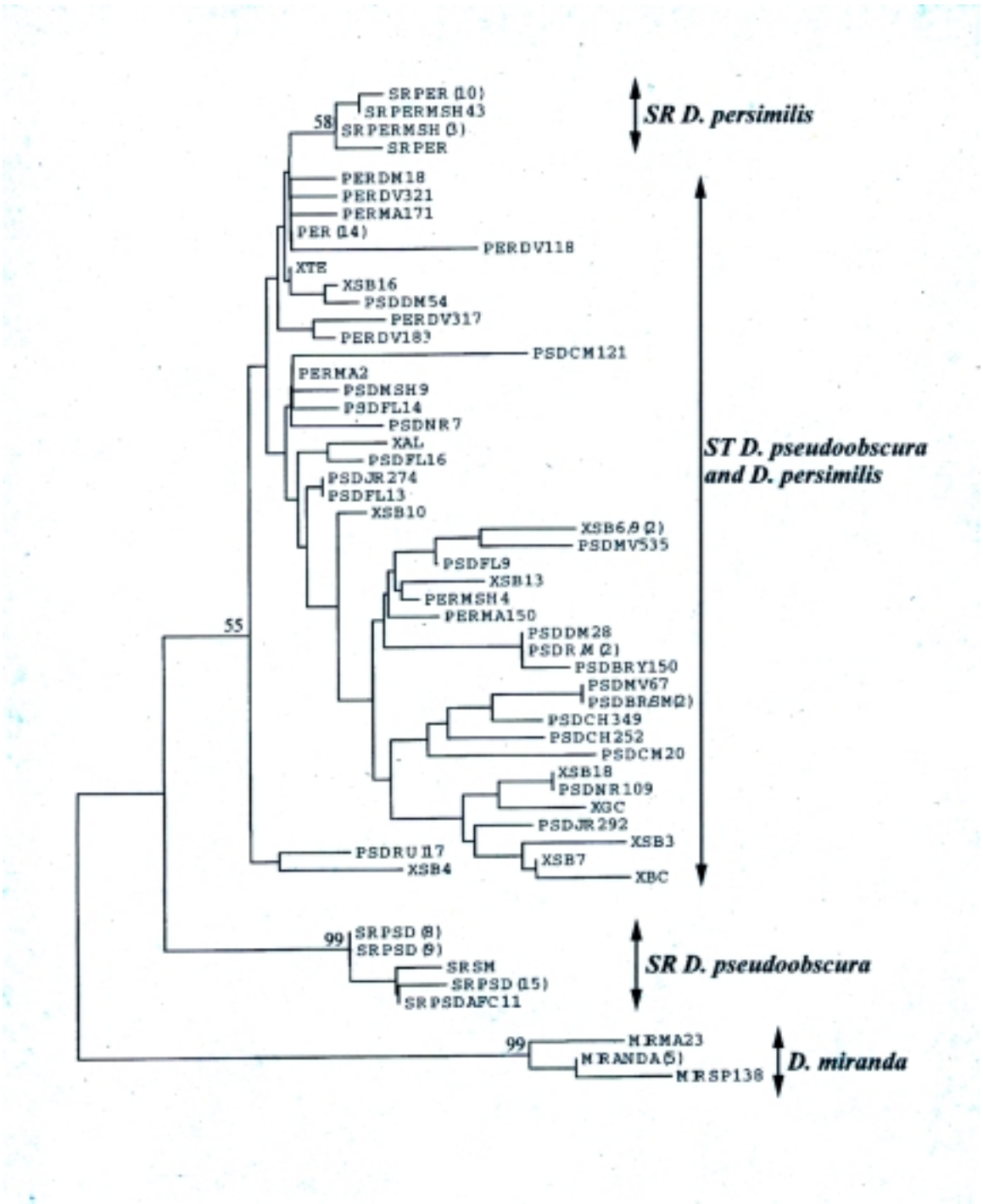

f
mg Est-5
s
D. pseudoobscura ST X
f
mg Est-5
s
D. pseudoobscura SR X
f
mg Est-5
s
D. persimilis ST X
f
mg Est-5
s
D. persimilis SR X

Figure 2. Phylogenetic tree of the *esterase-5* sequenced region from the taxa in table 1, constructed by neighbor joining using MEGA. Identical sequences are condensed with the number of taxa in ( ). The percentage of times each branch was joined together out of 500 bootstrap replicates is located above the supporting branch. Arrows indicate the chromosomal arrangement the clusters represent.


SRPER (10)
SRPERMSH43
58
SRPERMSH (3)
SRPER
PERDM18
PERDV321
PERMA171
PER (14)
PERDV118
XTE
XSB16
PSDDM54
PERDV317
PERDV183
PSDCM121
PERMA2
PSDMSH9
PSDFL14
PSDNR7
XAL
PSDFL16
PSDJR274
PSDFL13
XSB10
XSB6 (2)
PSDMV535
PSDFL9
XSB13
PERMSH4
PERMA150
55
PSDDM28
PSDRM (2)
PSDBRY150
PSDMV67
PSDBRSM (2)
PSDCH349
PSDCH252
PSDCM20
XSB18
PSDNR109
XGC
PSDJR292
XSB3
PSDRU117
XSB4
XSB7
XBC
SR D. persimilis
ST D. pseudoobscura and D. persimilis
99
SRPSD (8)
SRPSD (9)
SRSM
SRPSD (15)
SRPSDAFC11
SR D. pseudoobscura
99
MRMA23
MRANDA (5)
MRSP138
D. miranda